ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 40. SUPPLEMENTUM 136.

From the Department of Histology,
University of Lund, Sweden

PARAVENTRICULAR NUCLEUS AND PITUITARY GLAND

BY

HANS OLIVECRONA

LUND 1957

LUND
HÅKAN OHLSSONS BOKTRYCKERI
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INTRODUCTION

In recent years much interest has been focused on problems bearing on the hypothalamic neural control of pituitary function. The importance of various hypothalamic regions for the secretion of hypophysial hormones can be studied by determining the location and extent of those areas, the destruction of which will result in diminished formation and secretion of individual hormones by the pituitary, while their stimulation in the intact animal will be followed by an increased release of these hormones from the gland.

The two main fibre tracts forming part of the total nerve fibre connections between the hypothalamus and the neural division of the pituitary gland originate in the supraoptic and paraventricular nuclei, and are termed the supraopticoneurohypophysial and paraventriculoneurohypophysial tract, respectively.

In the experiments on hypothalamic lesions as yet described, no attempt has actually been made to restrict the lesions to only one of these fibre systems. It is therefore not known whether the supraoptic and paraventricular nuclei with their nerve fibres serve a common purpose or whether the separation of the two nuclei in higher vertebrates is also attended with a differentiation in function. Achieving degeneration of one of the two above-mentioned fibre systems with minimal involvement of the other would require destruction of its nuclei of origin, because when, as has generally been done, the lesions are placed basally in front of the median eminence, nerve fibres belonging to both fibre systems will be involved in the lesions.

The *purpose* of the present investigation was to analyze the effect of a loss of the paraventriculoneurohypophysial fibre system, produced by localized electrolytic lesions in the region of the paraventricular nuclei, on the function and hormonal content

of the posterior pituitary gland. Further, it was considered of interest to study also the effect of such lesions on the secretion of some of the anterior pituitary hormones, because experiments have been reported indicating the paraventricular region to be concerned with the control of some of the anterior pituitary functions.

Chapter I

GENERAL TECHNIQUE

Albino rats, inbred for 8 generations, were used as experimental animals. Inbreeding reduces individual anatomic variations: this is of importance since the sites of the various hypothalamic regions are referred to in relation to the point of intersection between the coronal and sagittal sutures. At the time of operation the animals weighed between 130 and 230 grams. Both male and female rats were used.

LESION TECHNIQUE

The hypothalamic lesions were performed with an instrument designed by HILLARP (1947). Fig. 1 gives a total view of the instrument, which consists of 3 principal parts, *viz.* the head holder, the electrode guide, and the pantograph pencil with its framework.

The head holder, which is detachable, is furnished with 3 fixation pins, 1 rostral and 2 caudal, the latter being situated in one and the same frontal plane. With the aid of the rubber-covered metal tongue that is placed in the mouth of the animal, the head can be firmly screwed up against the 3 fixation pins, which will hold the head perpendicular to the electrode (Fig. 2).

The adjustment of the electrode in the horizontal plane is based on the pantograph principle, and the instrument is so constructed that the electrode moves one tenth of the distance traversed by the pantograph pencil. This pencil is moved within a co-ordinate system drawn on millimeter squared paper where the zero point is represented by that position of the pencil where the electrode is located above the tip of the rostral fixation pin of the head holder. The electrode, made of nichrome wire of 0.3 mm diameter, is insulated with shellac except for the blunt

tip, which is free from insulation for a distance of 0.4 mm. The electrode represents the anode. The metal tongue in the mouth of the animal serves as the indifferent electrode.

The lesions were produced by a direct current of low amperage passing through the electrode, the size of the lesions depending on the strength and duration of the current. A current of 0.5 mA for 20 seconds proved sufficient to produce complete destruction of the paraventricular nuclei with at most slight involvement of the surrounding structures.

Under ether anaesthesia a midline incision was made through the skin over the skull. After removal of the periosteum the head was fixed in the holder so that the rostral fixation pin was located at the point of intersection of the coronal and sagittal sutures. Under a binocular microscope ($\times$ 15) a hole was then drilled through the bone down to the dura on each side of the midline. In operations intended to destroy the paraventricular nuclei the holes were placed close to the sagittal suture about 2 mm behind a frontal plane through the rostral fixation pin. The two holes were then widened medially until all bone between them had been removed without injure to the sagittal sinus. The dura was opened with a small needle. After the haemorrhage, which was minimal, had ceased, the animal was mounted in the instrument by the head holder being placed in position.

For destruction of the paraventricular nuclei a lesion was placed on each side of the third ventricle. The electrode was passed down at a point 2.0 mm behind the rostral pin and 0.5 mm lateral to the midline. The electrode was first allowed to sink by its own weight down through the brain to the base of the skull and then raised 1.0 mm, which proved to be the most suitable height above the base of the skull for lesions intended to destroy the paraventricular nuclei.

In attempts to destroy the elongated supraoptic nuclei use was made of 4 lesions, 2 on each side of the midline. The rostral lesions were placed 1.3 mm, and the caudal lesions 1.7 mm, behind the rostral fixation pin of the head holder, the former somewhat more medially than the latter, the distance from the midline being 1.2 and 1.4 or 1.6 mm, respectively. The most suitable height above the base of the skull was found to be 0.8 mm.

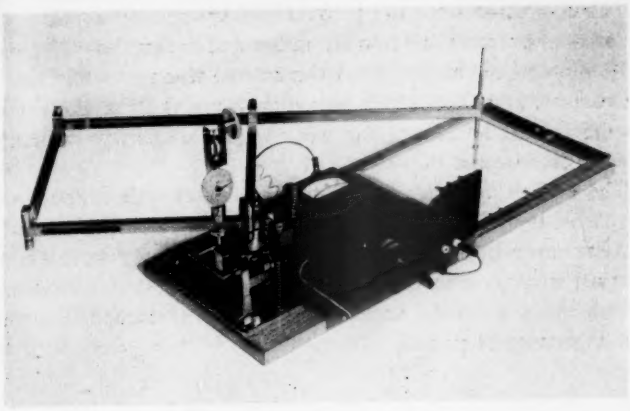


Fig. 1. Total view of the lesion instrument with the movable pantograph pencil and its co-ordinate system on the right. (From HILLARP, N.-Å., 1947).

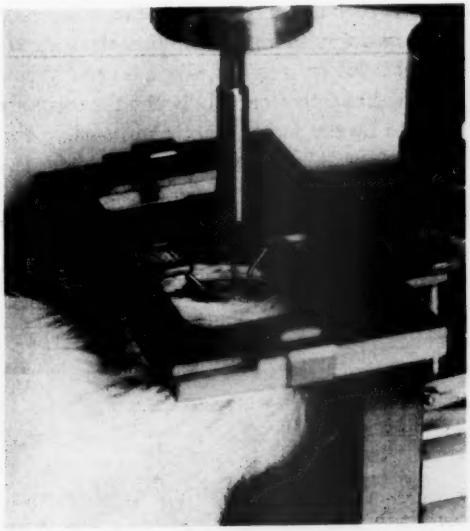


Fig. 2. Animal mounted in the head holder with the three fixation pins in position. (From HILLARP, N.-Å., 1947).

The co-ordinates for the paraventricular and supraoptic nuclei, respectively, were the same for males as for females.

At the end of the operation the animal was removed from the instrument, and the incision closed with metal clips. To facilitate uncomplicated healing of the wounds, the animals were kept for 1 week in separate cages.

The overall operative mortality from electrolytic lesions in the region of the paraventricular nuclei was about 30 per cent.

After operation the animals were often very irritable and reacted strongly even to slight stimuli.

The condition of the animals was carefully watched throughout the experimental period.

REPRODUCIBILITY

In investigations based on localized destruction of a given region of the central nervous system it is of great importance that the technique used admits of serial reproduction of comparable lesions. In most of the experimental series in which destruction of the paraventricular nuclei was intended, complete destruction was obtained in about 60 per cent of the animals. Variation in the position of the point of intersection between the coronal and sagittal sutures appears to be the factor mainly responsible for inaccurate placing of the lesions. However, it proved an advantage that a number of animals in each series showed lesions in the vicinity of the paraventricular nuclei instead of complete destruction of these nuclei, since these animals could serve as controls of the operation effect as well as of the significance of the surrounding structures for the results obtained.

At the conclusion of the experiment the animals were killed by decapitation under light ether anaesthesia.

HISTOLOGIC PROCEDURES

The whole brain was fixed in Carnoy's solution for 24 hours, after which a block of tissue containing the hypothalamic region was cut out, dehydrated and embedded in paraffin with methyl-

benzoate-celloidin as intermedium. Serial sections were cut in the frontal plane at 10 μ, every fourth section being mounted and stained with gallocyaninchrome alum according to EINARSON (1932).

The hypophysis was weighed and fixed in Susa for 1½ hour. After dehydration it was embedded in paraffin and cut serially at 5 μ, in the horizontal plane. Every third section was mounted, and the sections were stained with Azan.

The thyroids, adrenals and ovaries were weighed, fixed in formalin 1 : 3, embedded in paraffin, sections cut at 10 μ, and stained with haematoxylin and eosin.

Other histologic methods used for special studies will be found described in the appropriate chapters.

STATISTICAL METHODS

In the statistical treatment of the data the procedures described by DAVIES (1949) were adopted.

The following formulae were used:

$$\text{The mean value } \bar{x} = \frac{\Sigma x}{n}$$

were x denotes single observations and n the number of observations.

The standard error of the mean:

$$\text{S. E.}(\bar{x}) = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n(n-1)}}$$

To determine whether the difference between two mean values, x_1 and x_2, was significant, the number of observations being n_1 and n_2 respectively, the *t*-test was used:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\text{S. E.}(\bar{x}_1 - \bar{x}_2)}$$

where the standard error of the difference:

$$\text{S. E.}(\bar{x}_1 - \bar{x}_2) = \sqrt{\frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2} \cdot \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

In the treatment of pairs of observations, *i. e.* one for the right and one for the left side, the difference between the two sides was analyzed according to the difference method:

$$\bar{d} = \frac{\Sigma d}{n}$$

where d denotes difference between right and left side in the successive pairs and n the number of pairs.

The t-value was then calculated according to the following formula:

$$t = \frac{\bar{d}}{\text{S. E.}(\bar{d})}$$

$$\text{S. E.}(\bar{d}) = \sqrt{\frac{\Sigma(d-\bar{d})^2}{n(n-1)}}$$

When P was less than 0.01, the differences were regarded as significant.

Chapter II

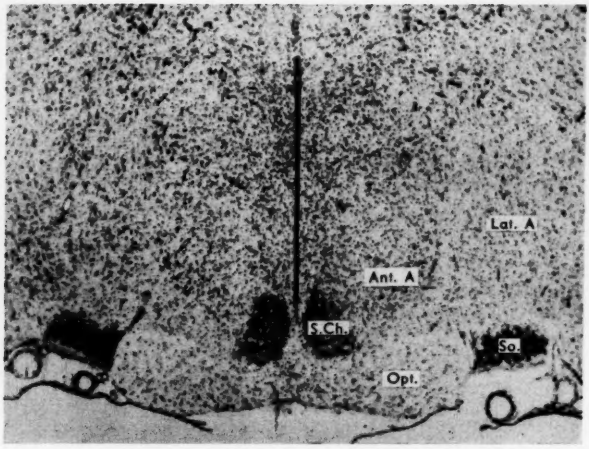
ANATOMIC NOMENCLATURE

The generally accepted nomenclature, suggested by RIOCH, WISLOCKI & O'LEARY (1940), will be used in discussions of the detailed anatomy of the hypothalamus and pituitary gland.

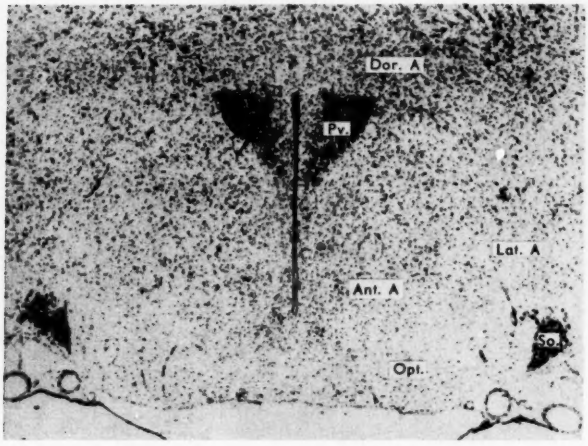
Fig. 3 shows transverse sections through the hypothalamus of the normal rat.

Abbreviations used in Fig. 3 a–d:

Ant. A	Anterior hypothalamic area.
Dor. A	Dorsal hypothalamic area.
D.M.	N. dorsomedialis.
Hyp.st.	Hypophysial stalk.
Lat. A	Lateral hypothalamic area.
M.em.	Median eminence.
Opt.	Optic chiasma.
Pe.arc.	N. periventricularis arcuatus.
Pv.	N. paraventricularis.
S.Ch.	N. suprachiasmaticus.
So.	N. supraopticus.
V.M.	N. ventromedialis.

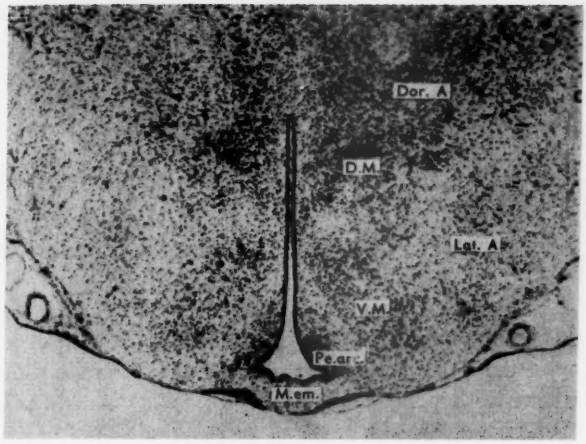


3 a. Section at the level of the suprachiasmatic nuclei.

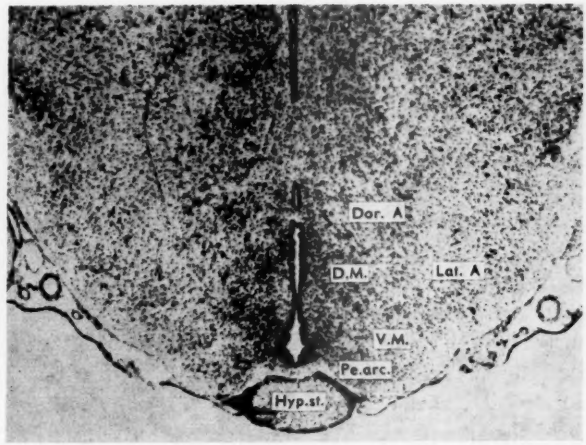


3 b. Section at the level of the paraventricular nuclei.

Fig. 3 a–d. Transverse sections through the hypothalamus of the normal rat. Gallocyanin stain. × 25.



3 c. Section at the level of the median eminence.



3 d. Section at the level of the hypophysial stalk.

Chapter III

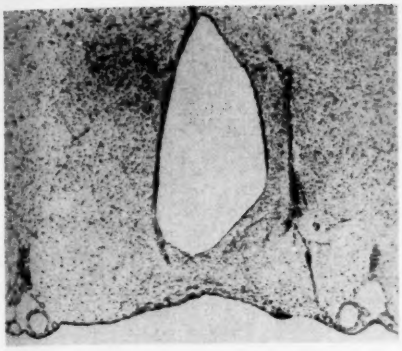
DESCRIPTION OF TYPICAL LESIONS DESTROYING THE PARAVENTRICULAR NUCLEI

The lesions extended continuously from the level of the suprachiasmatic nuclei to the region above the anterior or middle portion of the median eminence. They were thus entirely situated in the anterior hypothalamus. The lesions were located medially close to the ventricular system, causing destruction of the periventricular tissue above and immediately behind the posterior end of the optic chiasma. Figs. 4 and 5 show transverse sections through hypothalami from animals in which the lesions had produced complete destruction of the paraventricular nuclei.

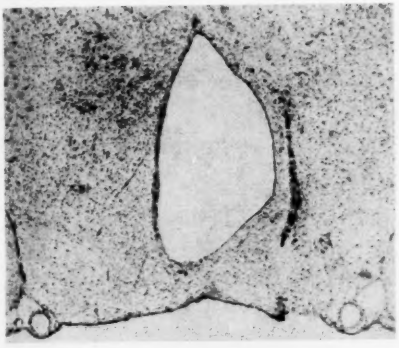
In their extension along the ventricular wall the lesions anteriorly destroyed part of the medial portion of the anterior hypothalamic areas, sometimes also involving part of the suprachiasmatic nuclei. In the region above the optic chiasma the transverse extent of the lesions corresponded approximately to the middle third of the width of the chiasma. The supraoptic nuclei were thus not directly involved by the lesions. As a consequence of the destruction of the periventricular tissue a varying degree of widening of the third ventricle was obtained.

At the level of the region of the paraventricular nuclei, simultaneously with the complete destruction of these nuclei, the lesions involved the medial portions of the anterior hypothalamic areas, the dorsomedial nuclei and the dorsal hypothalamic areas.

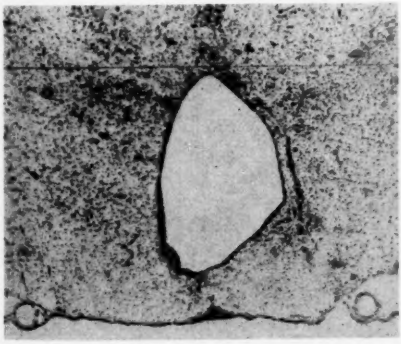
Behind the paraventricular nuclei and above the region of the median eminence the lesions destroyed the medial portions of the ventromedial nuclei, the dorsomedial nuclei and the dorsal hypothalamic areas. With but very slight tissue destruction the lesions terminated above the anterior or middle portion of the median eminence without directly involving this latter structure.



4 a. Section at the level of the optic chiasma.

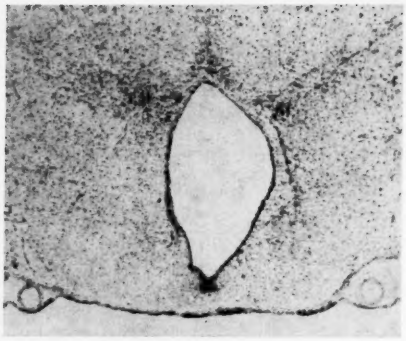


4 b. Section at a level through the anterior portion of the paraventricular region.

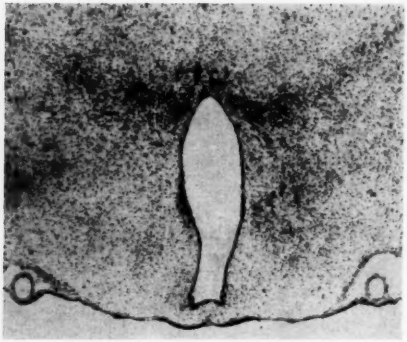


4 c. Section at a level through the posterior portion of the paraventricular region.

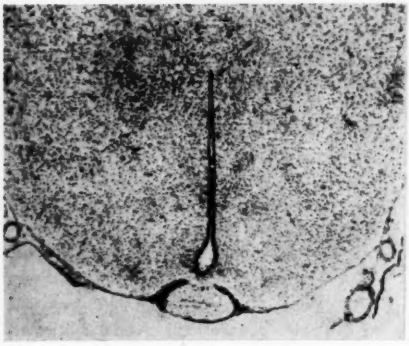
Fig. 4 a–f. Transverse sections through the hypothalamus of an animal with periventricular electrolytic lesions causing complete destruction of the paraventricular nuclei. Gallocyanin stain. × 19.



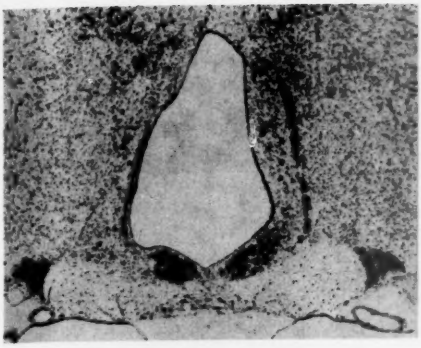
4 d. Section at a level in front of the median eminence.



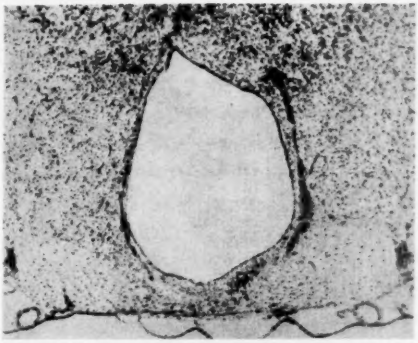
4 e. Section at a level through the anterior end of the median eminence.



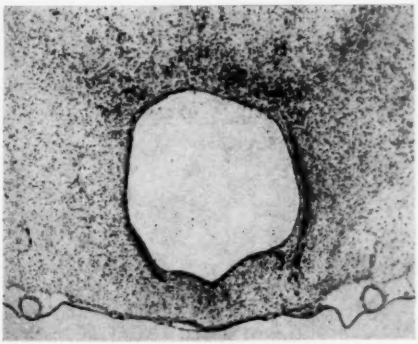
4 f. Section at the level of the hypophysial stalk.



5 a. Section at the level of the optic chiasma.

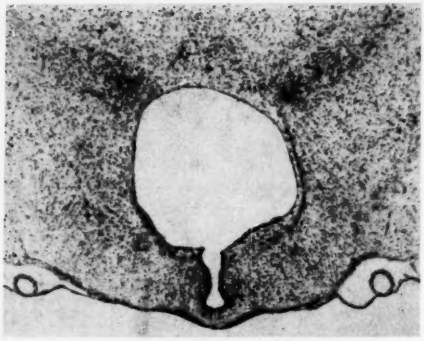


5 b. Section at a level through the anterior portion of the paraventricular region.

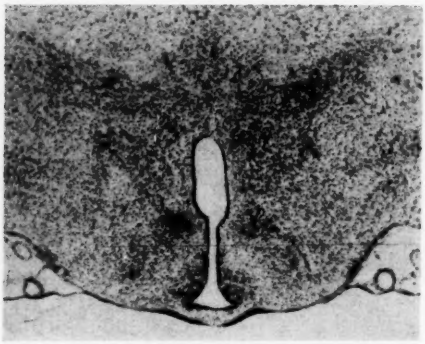


5 c. Section at a level through the posterior portion of the paraventricular region.

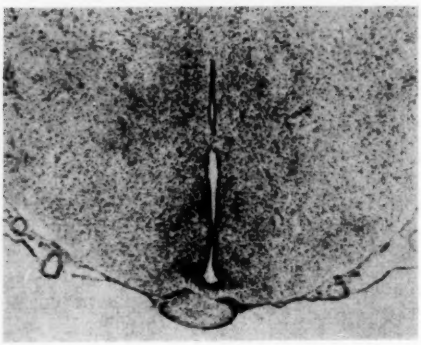
Fig. 5 a–f. Transverse sections through the hypothalamus of an animal with periventricular electrolytic lesions causing complete destruction of the paraventricular nuclei. Gallocyanin stain. × 19.



5 d. Section at a level through the anterior end of the median eminence.



5 e. Section at a level through the posterior end of the median eminence.



5 f. Section at the level of the hypophysial stalk.

Behind the optic chiasma the lesions caused slight or no destruction of the mediobasal tissue under the floor of the third ventricle. Only exceptionally did the lesions involve the base of the brain, apart from the narrow tracks left by the passage of the electrode.

The only nucleus or nuclear area that was completely destroyed by these lesions was the paraventricular nucleus. Technically, then, it was possible to study the effect of a loss of this nucleus and its fibre system.

In experiments aiming at a destruction of localized regions in the hypothalamus it is of great importance that the lesions can be limited to such an extent that they will achieve the desired effect with as slight an involvement as possible of the surrounding structures. With large and extensive destruction it is no longer possible to discuss the findings as a consequence of a loss of a given limited hypothalamic area, but simply as an effect of a loss of a hypothalamic neural control. The extent of the lesions depends on the strength and the duration of the current. In the present investigation, as mentioned above, the lesions in the region of the paraventricular nuclei were made by means of a current of 0.5 mA for 20 seconds. In this way it seemed possible to make less extensive electrolytic lesions than those usually reported. Thus, for example, McCann (1953) used 6.0 mA for 15 seconds, Mayer & Greenberg (1953) 1.5 mA for 15 seconds, Anand, Raghunath, Dua & Mohindra (1954) 1.0 mA for 15 seconds, and Greer (1955) 6.0 mA for 15 seconds for making hypothalamic lesions in the rat. The use of a current of such strength, however, must surely produce considerable tissue damage.

Chapter IV

ANATOMIC ANALYSIS OF THE PARAVENTRICULAR NUCLEUS AND ITS FIBRE SYSTEM

The nerve fibres running from the hypothalamus to the posterior pituitary gland form in the median eminence and infundibular stem the hypothalamoneurohypophysial tract, which consists of two main parts, the supraopticohypophysial tract, taken in the wider sense of the term, running in the ventral wall of the hypophysial stalk, and the tuberohypophysial tract in the dorsal wall.

The major portion of the nerve fibres running in the ventral wall of the hypophysial stalk originates in the magnocellular hypothalamic nuclei, the supraoptic and the paraventricular nuclei. In lower vertebrates these two nuclei are represented by one common nucleus, the preoptic nucleus (MEYER, 1935).

It is not known with certainty from which nuclei the tuberohypophysial tract originates, but they are believed to be situated in the tuberal region and in the region of the mammillary body.

The nerve supply to the neurohypophysis can be studied either by direct demonstration of the course of the nerve fibres from the hypothalamus to the infundibular stem, or indirectly by observation of the retrograde degeneration of the nuclei of origin, which occurs after interruption of the nerve fibres passing to the posterior pituitary gland.

CAJAL (1894), using silver impregnation, showed a large tract of unmyelinated nerve fibres running from the hypothalamus to the infundibular process in the rat. PINES (1925) and GREVING (1925, 1926) showed that some of the nerve fibres in the infundibular process originated in the supraoptic nuclei. GREVING called this fibre connection the supraopticohypophysial tract. These findings were confirmed by STENGEL (1926). GREVING (1928) also

observed nerve fibres originating in the paraventricular nuclei and passing in a ventral direction towards the supraoptic nuclei, but he could not state whether they terminated in relation to these nuclei or whether they joined the supraopticohypophysial tract. He called this fibre system the tractus paraventricularis cinereus. In a series of investigations ROUSSY & MOSINGER (1933 a and b, 1934, 1935) arrived at similar conclusions concerning the origin and course of the nerve fibres to the infundibular process. LARUELLE (1934) distinguished two portions in a paraventriculo-hypophysial fibre system, *viz.* a lateral fascicle corresponding to the tractus paraventricularis cinereus of GREVING, and a medial fascicle extending along the wall of the third ventricle towards the hypophysis. According to FISHER, INGRAM & RANSON (1938), the major part of the nerve fibres from the paraventricular nuclei, running in a ventrolateral direction, terminate in relation to the supraoptic nuclei, only a small portion joining the supraoptico-hypophysial tract. The major part of the numerous unmyelinated nerve fibres in the ventral wall of the infundibular stem should therefore originate in the supraoptic nuclei. INGRAM (1940) made similar observations in man.

The results of investigations using the chrome alum haematoxylin phloxine procedure of GOMORI agreed well with those made earlier with the use of silver impregnation (BARGMANN, 1949 a and b, BARGMANN & HILD, 1949, LAQUEUR, 1954). In addition to the nerve fibres running from the paraventricular nuclei in a ventrolateral direction towards the supraoptic nuclei and the proximal part of the supraopticohypophysial tract, LAQUEUR proved, in the dog, the existence of a direct passage of nerve fibres located behind the supraoptic nuclei and extending from the dorsocaudal portion of the paraventricular nuclei to the supraopticohypophysial tract in the region of the median eminence and proximal part of the infundibular stem.

The reaction of the cells in the supraoptic and paraventricular nuclei to various types of operations on the hypothalamoneuro-hypophysial system, has also been used for estimating to what extent nerve fibres running to the posterior pituitary gland originate in these nuclei. KARY (1924) and LEWY (1924) were the first to describe a retrograde degeneration of cells in the supraoptic

nuclei following destruction of the infundibular process. Numerous studies dealing with the anatomy of the hypothalamoneurohypophysial fibre system have confirmed this finding, and further shown that hypophysectomy or transection of the hypophysial stalk results in marked degeneration of the supraoptic nuclei with disappearance of 80 to 90 per cent of the cells (Maiman, 1930, Broers, 1932, Fisher, Ingram & Ranson, 1935, 1938, Ingram & Fisher, 1936, Hare, 1937, Magoun & Ranson, 1939, Rasmussen, 1940, Rasmussen & Gardner, 1940, Heinbecker & White, 1941, Hickey, Hare & Hare, 1941, Pickford & Ritchie, 1945, O'Connor, 1947, Heinbecker, White & Rolf, 1947, Bodian & Maren, 1951). This, then, implies that practically all the cells in the supraoptic nuclei send their axons to the neurohypophysis.

Qualitative microscopic examination alone of the paraventricular nuclei after hypophysectomy or transection of the hypophysial stalk has given varying results. Thus, Rasmussen (1940) found no regular nuclear changes suggesting an actual cell loss after these operations. In connection with experiments on the supraopticohypophysial tract other investigators, however, observed signs of moderate atrophy of the paraventricular nuclei (Broers, 1932, Fisher, Ingram & Ranson, 1935, 1938, Ingram & Fisher, 1936, Magoun & Ranson, 1939, Heinbecker & White, 1941, Heinbecker, White & Rolf, 1947).

Reliable information on the reaction of the cells in the paraventricular nuclei to section of the hypothalamoneurohypophysial fibre connections, however, cannot be obtained without a quantitative determination of the number of cells within the nuclei. The first to perform such an analysis was Frykman (1942), who, after hypophysectomy, noted a loss of about 35 per cent of the magnocellular cells in the paraventricular nuclei. Other authors have reported more marked atrophy of the nuclei after similar operations. Thus, after intervention on the supraopticohypophysial fibre system Pickford & Ritchie (1945) found an 84 per cent decrease in the number of cells in the paraventricular nuclei, while O'Connor (1947) observed a reduction of 69 per cent after hypophysectomy and of 80 per cent after division of the supraopticohypophysial tract in the median eminence. Extirpation of half of the infundibular process with

preservation of the major part of the pars distalis caused 63 per cent reduction in the number of magnocellular cells in the paraventricular nuclei, while the cell loss at the same neurohypophysial remnant but after complete removal of the pars distalis was 85 per cent (BODIAN & MAREN, 1951).

Hypophysectomy or transection of the hypophysial stalk thus produces marked retrograde degeneration of the supraoptic nuclei with a loss of about 80 to 90 per cent of their cells. It seems probable that the nerve fibres in the hypothalamoneurohypophysial fibre system terminate in all three parts of the neurohypophysis. It has therefore been assumed that the axons from those cells in the supraoptic nuclei that persist after these types of operations terminate in relation to the median eminence, which remains intact.

Experiments in which hypophysectomy or transection of the hypophysial stalk are used for investigating the importance of the paraventricular nuclei for the innervation of the infundibular process, have thus given varying results as regards the occurrence of retrograde degeneration of the cells in these nuclei. Even if such a retrograde cell reaction does occur after these operations, it does not necessarily mean that the axons from the paraventricular nuclei run to the neurohypophysis, since transneuronal degeneration might be present if the axons from the paraventricular nuclei end in relation to the cells of the supraoptic nuclei. Such a course of the axons was, as pointed out above, assumed by FISHER, INGRAM & RANSON (1938). It therefore appeared desirable to analyze the course and the connections of the fibre system extending from the paraventricular nuclei. It was further considered of interest to determine whether the supraoptic nuclei send nerve fibres not only to the neurohypophysis, but also to the paraventricular nuclei. Since the pars intermedia of the pituitary gland is innervated from the infundibular process (see Discussion), its morphologic reaction to hypothalamic lesions was also studied.

The investigations were planned as a quantitative morphologic analysis along the following lines:

1. Quantitative determination of the reaction of the paraventricular nuclei to transection of the neural stalk, which had been effective in producing a retrograde degeneration of the supraoptic nuclei.

2. Analysis of the intrahypothalamic course of the fibre system from the paraventricular nuclei performed indirectly by quantitative determination of the reaction of the paraventricular nuclei to electrolytic lesions in various regions of the hypothalamus.
3. Quantitative determination of the transverse sectional area of the infundibular stem, and of the volume of the infundibular process and intermediate lobe after loss of the fibre system from the paraventricular nuclei following their complete destruction.

METHODS

Transection of the neural stalk. – After parapharyngeal approach the hypophysial stalk was sectioned extracapsularly according to the technique described by BROLIN (1943). Marked degeneration of the supraoptic nuclei, observable as a pronounced falling out of cells, was taken as a criterion of successful division of the neural stalk.

Quantitative determination of magnocellular cells. – The magnocellular cells of the paraventricular and supraoptic nuclei were counted under the high dry objective of a binocular microscope. The magnification was 420 ×. These cells are characterized by a peripheral arrangement of the Nissl substance, by an eccentrically located nucleus with a large, prominent nucleolus, and by a perinuclear area free from Nissl substance. The individual cells were carefully examined and only typical magnocellular cells containing a distinct nucleolus were counted. An ocular micrometer disc was used in one of the eye-pieces of the microscope, giving a square field, which in its turn was divided into nine smaller squares. In every section all the cells of typical appearance belonging to the magnocellular hypothalamic nuclei were counted, and with the aid of a mechanical stage each nucleus was systematically examined throughout its entire extent.

The total number of cells in a nucleus was obtained by multiplying the number counted by 4. In sections of the thickness used the error involved by a cell being counted twice because of splitting of the nucleolus is not great, and JONES (1937) showed that, if only cells with distinct nucleoli are counted, the error due to such splitting is less than 3 per cent.

It was not difficult to delimit, from the surrounding nuclear areas, the supraoptic nuclei with their typical, densely packed magnocellular cells with only slight admixture of cells of other types. Therefore, neither in the normal animals nor in those with marked atrophy of the nuclei did the counting of the cells present any difficulties. No counts were made of the cells in the nucleus supraopticus diffusus, representing the caudal extension of the nucleus lying basally along the ventral surface of the tuber cinereum.

The situation is quite different as regards the paraventricular nuclei. Determination of the boundaries of these nuclei offered considerable difficulty, which might at least partially explain the varying degree of degeneration reported as occurring in the paraventricular nuclei after hypophysectomy or transection of the hypophysial stalk.

The paraventricular nucleus begins and ends fairly abruptly, and is built up of two morphologically distinct parts, the nucleus paraventricularis magnocellularis and the nucleus paraventricularis parvocellularis. The magnocellular cells in the paraventricular nucleus are of the same type and have the same appearance as the cells in the supraoptic nucleus. Along the margins of the nuclear mass at the anterior and especially at the posterior pole the typical magnocellular cells are intermingled with large, fairly intensely stainable cells, at the posterior extremity belonging to the posterior hypothalamic area. However, these cells take on the stain less intensely than the typical magnocellular cells, and the Nissl substance is scattered all over the cytoplasm. Another factor making identification of the magnocellular cells difficult is the intermingling of cells present at the ventromedial border of the magnocellular portion of the paraventricular nucleus, this region containing not only the magnocellular cells but also small cells belonging partly to the parvocellular portion of the nucleus and partly to the periventricular system. This, then, makes the outline of the magnocellular portion of the paraventricular nucleus less sharp, which in its turn renders the quantitative analysis of the nucleus more difficult than that of the supraoptic nucleus. The lack of agreement between results obtained in investigations of the reaction of the paraventricular nuclei after hypophysectomy

or transection of the hypophysial stalk might therefore be explained by differences in the extent of inclusion of cells of borderline morphology, located at the anterior, posterior, and medial margins of the magnocellular portion of the nucleus.

From the anterior part of the supraoptic nucleus an irregular chain of scattered magnocellular cells can be seen extending in a dorsal direction and joining the lateral border of the paraventricular nucleus, thus forming a bridge between the two nuclei. In the present work these cells, which BODIAN & MAREN (1951) took together under the common name of the pars lateralis of the paraventricular nucleus, were not included at the quantitative determinations of the number of cells in the magnocellular portion of the paraventricular nucleus.

Silver impregnation. – In a series containing both normal animals and animals with complete destruction of the paraventricular nuclei an attempt was made to analyze the intrahypothalamic course of the axons from these nuclei, and further to determine the number of axons in the infundibular stem. After removal of the calvarium the entire head was fixed in Carnoy's solution. The hypothalamic region and the hypophysis were cut out *en bloc*, embedded in paraffin and cut serially either in the sagittal or in the frontal plane at 10 μ, every fourth section being mounted. The sections were treated according to BODIAN's silver technique (BODIAN, 1936). On comparing a number of different fixing fluids it was found that the best results of the silver impregnation were obtained after fixation in Carnoy's solution. This fixing fluid is thus preferable to the originally recommended procedure in which use is made of fixation by perfusion with 80 per cent alcohol.

Quantitative measurements of the infundibular stem, infundibular process and intermediate lobe. – For the quantitative determination of the transverse sectional area of the infundibular stem and of the volume of the infundibular process and intermediate lobe, sections were projected on a paper at a magnification of 100 times, and the outlines of the relevant structures were traced. The area of the surface traced was then determined planimetrically.

The infundibular stem was traced from five consecutive,

mounted sections beginning with that section in which the hypophysial stalk first appeared as a separate structure. The pars tuberalis of the adenohypophysis and the central cavity of the hypophysial stalk were not included in the measurements. The areas obtained by planimetry were added, and the sum divided by 5.

The infundibular process and intermediate lobe were traced from every third section mounted throughout the entire extent of these lobes. The areas of the two lobes in the traced sections were determined separately, and expressed in mm^2. The volume of the respective lobes was then determined by multiplying the sum of the individual areas by 0.045, which was the distance in millimeters between two consecutively traced sections.

RESULTS

NORMAL ANIMALS

In normal animals the length of the supraoptic nucleus in the anteroposterior direction was found to be 1.4 to 2.1 mm (mean 1.8 mm). The number of cells in the nucleus varied between 5 052 and 8 288 with a mean of 6 834 cells. The mean number of cells in the combined right and left supraoptic nuclei was $13\,668 \pm 483$ cells (Table 1). No difference was found between the right and left sides in the same animal or between males and females.

These figures agree well with the results of RASMUSSEN (1940) and BODIAN & MAREN (1951) in their determinations of the number of cells in the supraoptic nucleus of the rat. Thus, in a series of 6 animals RASMUSSEN found the number of cells in the nucleus to be about 7 000, and BODIAN & MAREN, likewise in 6 animals, found for the combined right and left nuclei a mean of 14 740 cells. MERRICK (1941, 1944) reported somewhat higher values, the total number of cells in the supraoptic nucleus in his series ranging from 7 605 to 9 186, with a mean of 8 419. He also showed that in the rat the number of cells in the supraoptic nucleus does not vary with age or sex. It seems probable that this also holds for the paraventricular nucleus.

In normal animals the length of the paraventricular nucleus in the

Table 1. *Number of cells in the supraoptic nuclei in intact animals.*

Rat No.	Sex	Body wt g	Number of cells		
			Right supraoptic nucleus	Left supraoptic nucleus	Combined supraoptic nuclei
N 151	♂	285	5180	5052	10232
152	♂	250	6296	6304	12600
153	♂	250	7096	7160	14256
154	♂	270	6740	6684	13424
155	♂	240	8128	8096	16224
156	♂	250	8288	8048	16336
147	♀	220	6784	6596	13380
148	♀	220	6748	6428	13176
149	♀	210	7384	7488	14872
150	♀	210	6840	7264	14104
157	♀	250	6448	6340	12788
158	♀	200	6220	6412	12632
Mean ± S.E.			6846 ± 242	6823 ± 245	13668 ± 483

t-test					
Subject of the test	Males	Females	Difference	Degrees of freedom	P
Right supraoptic nucleus	6955 ± 476	6737 ± 161	218 ± 502	10	> 0.1
Left supraoptic nucleus	6891 ± 470	6755 ± 201	136 ± 511	10	> 0.1
Combined supraoptic nuclei	13845 ± 945	13492 ± 348	353 ± 1006	10	> 0.1

Nor was any difference found between the right and left supraoptic nuclei, calculated according to the difference method. The following values were obtained:

$\bar{d} = 23.3$; S.E. $(\bar{d}) = 58.5$
Degrees of freedom = 11; $P > 0.1$

anteroposterior direction was found to be 0.44 to 0.84 mm (mean 0.60 mm). FRYKMAN (1942) found the length of the paraventricular nucleus in the rat to be fairly constant, lying between 0.65 and 0.70 mm, while BODIAN & MAREN (1951) gave a length of about 0.50 mm. The number of cells in the paraventricular nucleus varied between 1 052 and 1 544 with a mean of 1 335 cells. The mean

number of cells in the combined right and left paraventricular nuclei was 2 670 ± 72 cells (Table 2). Nor was any difference found here between the number of cells on the two sides in the same animal, or between males and females.

THE REACTION OF THE PARAVENTRICULAR NUCLEI ON TRANSECTION OF THE NEURAL STALK

Since retrograde degeneration of the cells in the supraoptic nuclei was taken as a criterion of successful division of the infundibular stem, the number of cells in these nuclei was determined parallel with the quantitative analysis of the paraventricular nuclei.

After transection of the infundibular stem the supraoptic nucleus showed marked atrophy with pronounced decrease in the number of cells, which varied between 860 and 2 960 with a mean of 1 785 cells. The mean number of cells in the combined right and left supraoptic nuclei was 3 569 ± 382 cells (Table 3). This is about 25 per cent of the number of cells found in the normal animals. There was thus a highly significant reduction which was of equal size on both sides. The length of the supraoptic nucleus was slightly less than in normal animals, varying between 1.2 and 1.9 mm (mean 1.5 mm).

The pronounced degeneration of the supraoptic nuclei thus showed that the nervous connections between the hypothalamus and the hypophysis had been divided, so that these animals were also well suited for a quantitative determination of the cells in the paraventricular nuclei after this operation. The magnocellular, dorsolateral portion of the paraventricular nucleus, also showing a marked loss of cells, however, retained its triangular shape as seen in frontal sections.

After division of the infundibular stem it became even more difficult than in normal animals to determine the boundaries between the paraventricular nucleus and the surrounding nuclear areas, especially at the posterior extremity, where the fairly large cells belonging to the posterior hypothalamic area, which had not undergone any demonstrable atrophy, were intermingled with the magnocellular cells of the paraventricular nucleus.

Also the length of the paraventricular nucleus was somewhat

Table 2. *Number of magnocellular cells in the paraventricular nuclei in intact animals.*

Rat No.	Sex	Body wt g	Number of cells		
			Right paraventr. nucleus	Left paraventr. nucleus	Combined paraventr. nuclei
N 151	♂	285	1052	1120	2172
152	♂	250	1192	1116	2308
153	♂	250	1544	1348	2892
154	♂	270	1448	1488	2936
155	♂	240	1480	1444	2924
156	♂	250	1316	1344	2660
147	♀	220	1456	1264	2720
148	♀	220	1504	1332	2836
149	♀	210	1384	1228	2612
150	♀	210	1272	1476	2748
157	♀	250	1352	1468	2820
158	♀	200	1204	1212	2416
Mean ± S.E.			1350 ± 43	1320 ± 39	2670 ± 72

t-test					
Subject of the test	Males	Females	Difference	Degrees of freedom	P
Right parav. nucleus	1339 ± 77	1362 ± 46	−23 ± 90	10	>0.1
Left parav. nucleus	1310 ± 65	1330 ± 48	−20 ± 81	10	>0.1
Combined parav. nuclei	2649 ± 137	2692 ± 64	−43 ± 151	10	>0.1

Nor was any difference found between the right and left paraventricular nuclei, calculated according to the difference method. The following values were obtained:

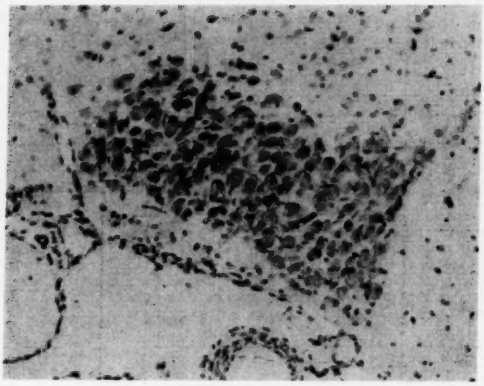
$\bar{d} = 30.3$; S.E. $(\bar{d}) = 37.6$
Degrees of freedom = 11; $P > 0.1$

less than in the normal animals, and varied between 0.32 and 0.64 mm (mean 0.43 mm). The number of magnocellular cells in the paraventricular nucleus varied in the animals with transection of the neural stalk between 88 and 516 with a mean of 319 cells. The mean number of cells in the combined right and left

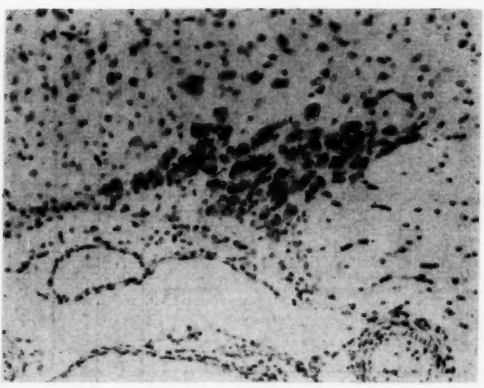
Table 3. *Number of cells in the supraoptic and paraventricular nuclei after transection of the infundibular stem.*

Rat No.	Sex	Days after op	Body wt g	Wt of both ovaries	Number of cells					
					Right supraoptic nucleus	Left supraoptic nucleus	Combined supraoptic nuclei	Right paraventr. nucleus	Left paraventr. nucleus	Combined paraventr. nuclei
St 1	♀	76	175	10.4	1568	1816	3384	408	368	776
6	♀	63	180	16.3	2316	2336	4652	436	500	936
10	♀	59	160	37.3	964	1124	2088	296	360	656
16	♀	57	155	9.6	2960	2628	5588	272	372	644
22	♀	52	160	29.9	1960	1852	3812	352	276	628
23	♀	53	150	34.1	1824	2032	3856	300	432	732
24	♀	52	145	10.1	1432	1292	2724	168	140	308
25	♀	52	125	10.9	2472	2344	4816	516	504	1020
27	♀	51	190	12.0	1452	1424	2876	244	232	476
40	♀	47	170	23.2	1036	860	1896	88	112	200
Mean ± S.E.					1798 ± 202	1771 ± 184	3569 ± 382	308 ± 40	330 ± 43	638 ± 81

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Combined right and left supraoptic nuclei	10099 ± 635	20	<0.001
Combined right and left paraventricular nuclei	2032 ± 108	20	<0.001

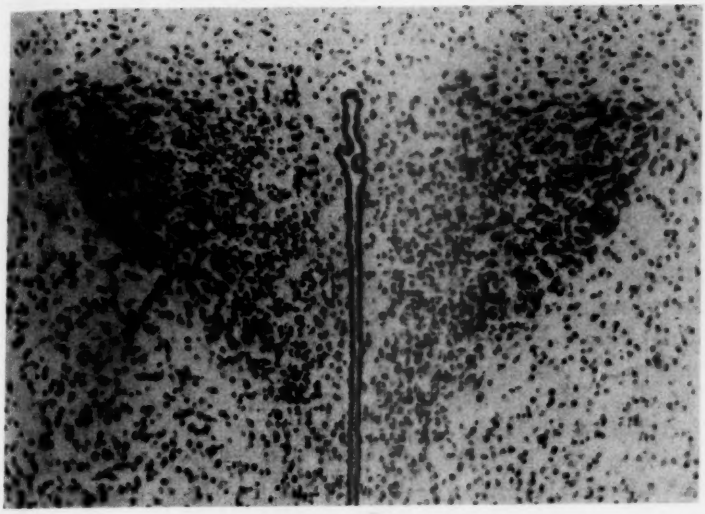


6 a. Normal supraoptic nucleus.

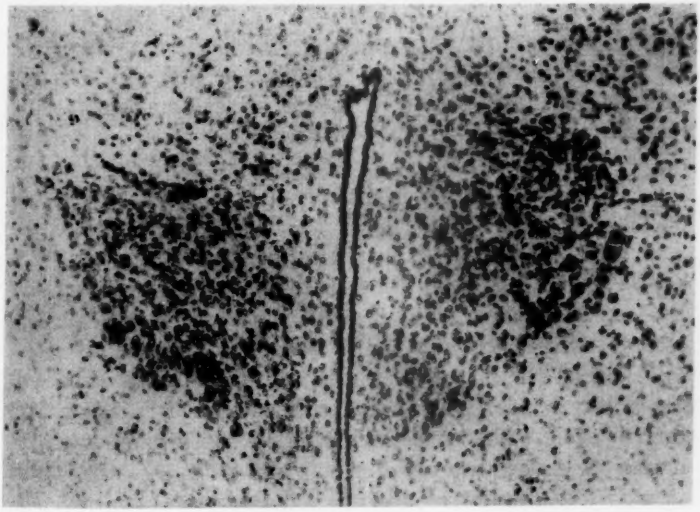


6 b. Supraoptic nucleus following transection of the infundibular stem.

Fig. 6 a–d. Transverse sections through the densest portions of the supraoptic and paraventricular nuclei of a normal animal (N 153) and of an animal with transection of the infundibular stem (St 23). Gallocyanin stain. × 113.



6 c. Normal paraventricular nucleus.



6 d. Paraventricular nucleus following transection of the infundibular stem.

paraventricular nuclei was 638 ± 81 cells (Table 3). Thus, also in the case of the paraventricular nuclei there was a highly significant reduction in the number of magnocellular cells after transection of the infundibular stem, only about 25 per cent surviving. Fig. 6 shows transverse sections through the densest portions of the supraoptic and paraventricular nuclei in normal animals as compared with animals with transection of the neural stalk.

No quantitative analysis was made of the parvocellular portion of the paraventricular nucleus because this part could not be clearly distinguished from the adjacent periventricular system. Examination of microscopic sections and serial photomicrographs showed, however, that transection of the infundibular stem causing pronounced loss of magnocellular cells in the paraventricular nucleus did not, apparently, affect the small, less intensely stainable cells belonging to the parvocellular portion of the nucleus. Since no quantitative analysis was made of the number of parvocellular cells, a possible reduction could not be excluded.

THE REACTION OF THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI ON HYPOTHALAMIC LESIONS

In an attempt to analyze the course of the fibre system from the paraventricular nucleus, a quantitative determination was made of the number of cells in this nucleus and also in the supraoptic nucleus after electrolytic lesions in various regions of the hypothalamus.

1. The number of cells in the supraoptic nucleus was counted on both sides in 18 animals with complete destruction of the paraventricular nuclei. The number of cells varied between 3 672 and 7 000 with a mean of 5 441 cells. The mean number of cells in the combined right and left supraoptic nuclei was 10 882 ± 302 cells (Table 4). This implies a statistically significant reduction by about 20 per cent as compared with the number of cells in the supraoptic nuclei of normal animals. Of the 36 nuclei counted from animals with complete destruction of the paraventricular nuclei, 11 showed a smaller number of cells than the lowest number found in any of the nuclei of the control material. The supraoptic nuclei thus showed a moderate degeneration after

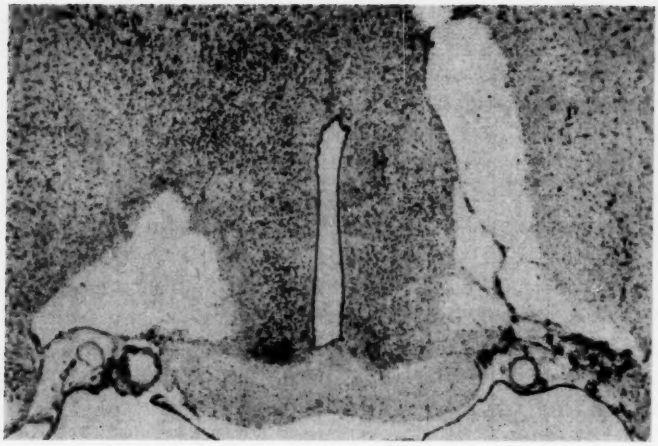
Table 4. *Number of cells in the supraoptic nuclei after complete destruction of the paraventricular nuclei.*

Rat No.	Sex	Days after op	Body wt g	Number of cells		
				Right supraoptic nucleus	Left supraoptic nucleus	Combined supraoptic nuclei
HL 270	♂	174	235	5248	4740	9988
311	♂	279	300	6392	4920	11312
313	♂	321	260	6296	5520	11816
332	♂	347	210	5368	5636	11004
336	♂	352	310	6008	6460	12468
337	♂	373	345	4668	5332	10000
341	♂	472	365	6340	5224	11564
346	♂	506	320	5180	4564	9744
349	♂	573	270	6512	6672	13184
363	♂	457	250	6248	5372	11620
366	♂	328	210	5492	4944	10436
397	♂	339	270	6444	7000	13444
405	♀	129	225	6072	3672	9744
407	♀	180	240	5788	5360	11148
415	♀	302	270	3980	5264	9244
436	♀	247	150	5124	4824	9948
485	♀	145	240	4904	4428	9332
486	♀	151	200	5220	4668	9888
Mean ± S.E.				5627 ± 169	5256 ± 193	10882 ± 302

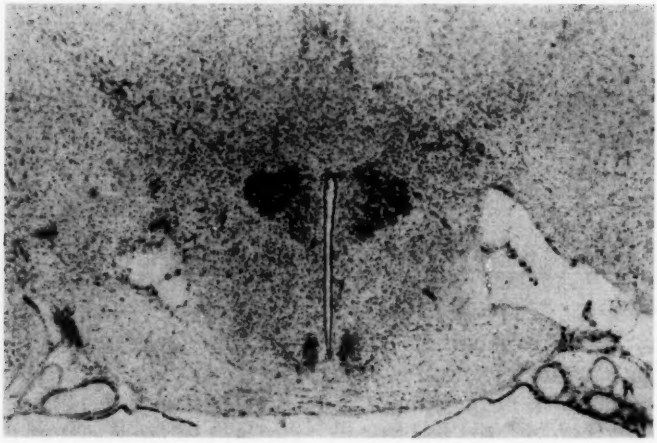
t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Combined right and left supraoptic nuclei	2786 ± 537	28	<0.001

destruction of the paraventricular nuclei. Microscopically, however, they appeared to be intact, and it was only the quantitative determination of the number of cells that revealed this atrophy.

2. After localized lesions had been made in the region of the supraoptic nucleus, counts were made both of the remaining cells in this nucleus, and of the number of cells in the paraventricular nucleus on the same side. Marked destruction of the supraoptic nucleus did not cause any reduction in the number of magnocellular cells in the paraventricular nucleus, which retained its

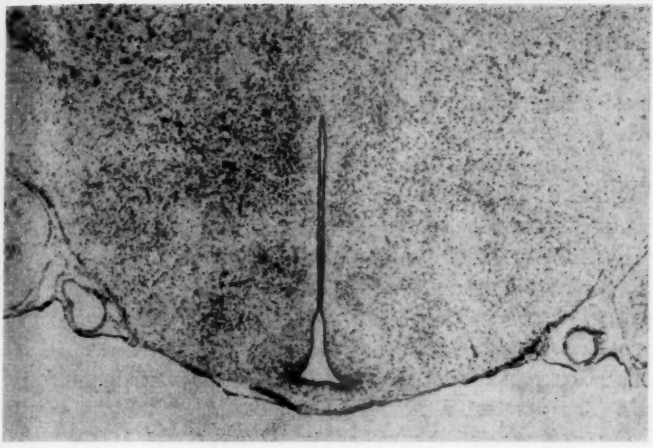


7 a. Transverse section at a level through the optic chiasma anteriorly to the paraventricular nuclei.



7 b. Transverse section at the level of the paraventricular nuclei.

Fig. 7 a–c. Normal appearance of the paraventricular nuclei in Rat HL 469, which has marked, direct destruction of the supraoptic nuclei. Gallocyanin stain. × 25.



7 c. Transverse section at the level of the median eminence.

Table 5. *Number of cells in the paraventricular nucleus after direct partial destruction of the supraoptic nucleus on the same side.*

Rat No.	Sex	Days after op	Body wt g	Number of cells	
				Supraoptic nucleus	Paraventricular nucleus
HL 455	♀	307	285	484	1232
459	♀	262	260	600	1224
465	♀	302	300	1908	1312
469	♀	99	260	2396 r. side	1256
469	♀	99	260	1220 l. side	1352
Mean ± S.E.				1322 ± 535	1275 ± 25

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
One supraoptic nucleus	5501 ± 508	15	<0.001
One paraventricular nucleus	75 ± 69	15	>0.1

normal triangular shape with a well developed dorsolateral portion (Table 5, Fig. 7). Thus, it does not seem probable that nerve fibres from the paraventricular nucleus end in relation to the supraoptic nucleus or, to any greater extent, pass in the immediate vicinity of that nucleus.

3. Lesions placed laterally to the paraventricular nucleus sometimes did not cause any reduction in the number of magnocellular cells, and this in spite of the lesions almost reaching the lateral border of the nucleus (Fig. 8). In other cases with lesions encroaching upon the lateral wing of the paraventricular nucleus, a varying loss of cells was observed in its magnocellular portion. When this reduction of cells was most marked, the nucleus contained about 60 per cent of the normal number of cells. The greatest difficulty in the evaluation of the reaction of the paraventricular nucleus to lesions situated immediately laterally to the nucleus was to exclude, with any degree of certainty, the existence of any direct lesion of the lateral wing. As a rule, this was not possible, so that the reduction in the number of cells recorded after laterally placed lesions might equally well be due to a partial destruction of the nucleus owing to a direct lesion. Those cases in which a normal number of magnocellular cells was found in the paraventricular nucleus after lateral lesions extending almost up to the nucleus were therefore of greater value for the analysis of the intrahypothalamic course of the fibre system from this nucleus than were lesions in which a subsequent reduction in the number of cells might have been, at least in part, due to a direct lesion of the lateral wing of the nucleus. Since the number of cells recorded in the paraventricular nucleus after lesions located close to the lateral border of the nucleus was in some cases normal and in others only slightly reduced, it seems probable that at least the major portion of the axons do not run in a lateral direction from the nucleus. If they did, they would have been destroyed by such lesions with subsequent retrograde degeneration of the cells in the nucleus. This opinion was further supported by the effect of the lesions placed slightly more laterally to the paraventricular nucleus, lying above the lateral third of the chiasma at the level of the nucleus, and in which a direct lesion of the magnocellular portion could

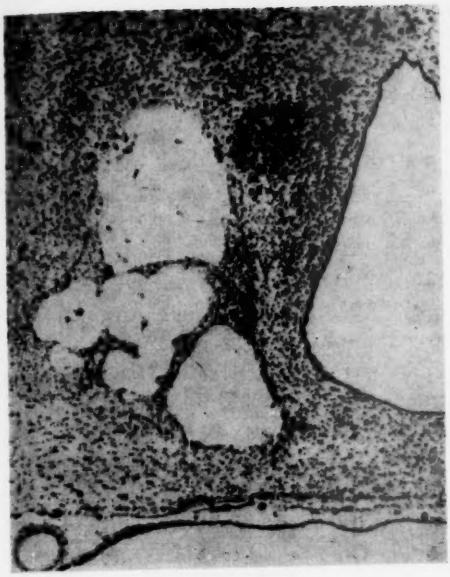
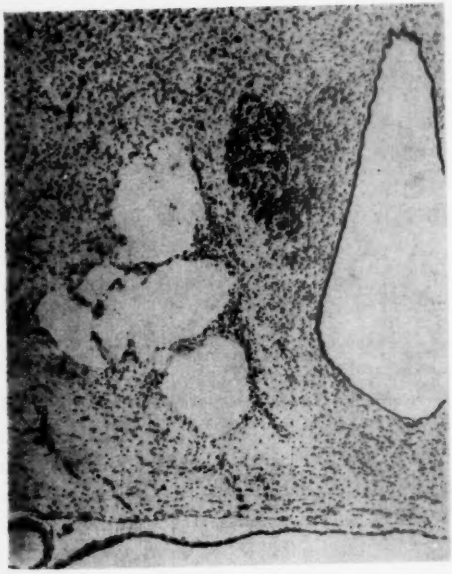


Fig. 8 a–b. Right paraventricular nucleus in Rat HL 438, which has a lesion located laterally to the nucleus. Gallocyanin stain. × 49.

8 a. Transverse section through the anterior portion of the paraventricular nucleus.



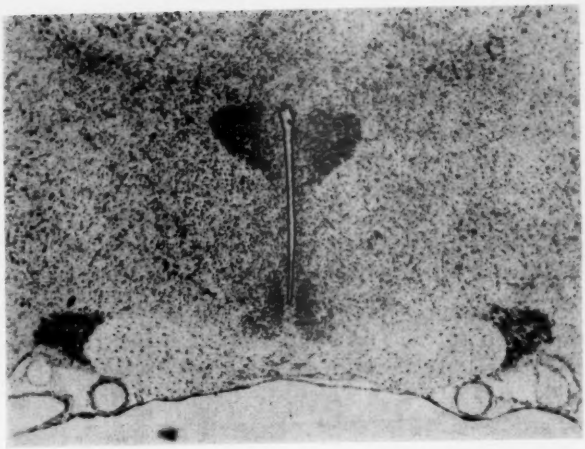
8 b. Transverse section through the posterior portion of the paraventricular nucleus.

be excluded with certainty. There was thus a band of intact tissue between the lesion and the paraventricular nucleus, which latter in these cases contained a normal number of magnocellular cells. The results of the quantitative analysis of the paraventricular nucleus after laterally placed lesions are summarized in Table 6.

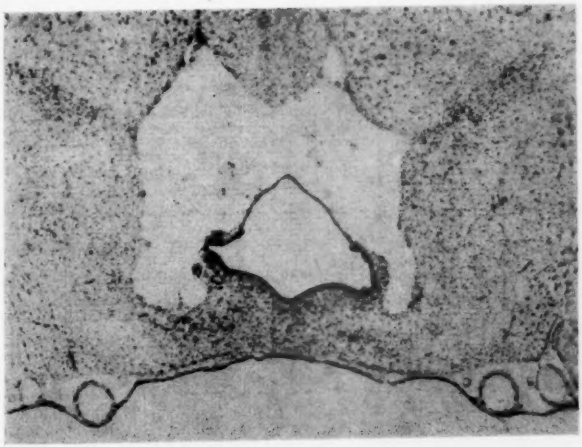
4. The axons from the paraventricular nucleus cannot run in a dorsal direction either, because lesions situated above and near the dorsal surface of the nucleus produced no retrograde degeneration resulting in distinct cell loss.

5. No reduction in the number of magnocellular cells in the nucleus was produced by hypothalamic lesions starting from immediately behind the paraventricular nucleus and extending in a caudal direction to the anterior end of the median eminence, initially leaving a small band of intact tissue close to the ventricular wall that was soon reached by the lesions, which then, just outside the median eminence, also extended down to the base of the brain. Such lesions, then, did not cause any interruption of the nerve fibres from the paraventricular nucleus (Table 7, Fig. 9).

The analysis of the extent of the total tissue destruction obtained by these electrolytic lesions in various regions of the hypothalamus showed that the route left for the nerve fibres from the paraventricular nucleus lies along a pathway first swerving gently outwards, then bending sharply downwards in a basocaudal direction. Then, just before the median eminence, it occupies a mediobasal position under the floor of the third ventricle and continues through the median eminence into the infundibular stem. That the nerve fibres follow such a course was further suggested by the reaction of the cells in the paraventricular nucleus after laterally placed lesions which extended into the mediobasal tissue under the floor of the third ventricle and continued so far caudally as also partly to destroy the anterior end of the median eminence. This type of lesion produced a pronounced reduction in the number of magnocellular cells in the paraventricular nucleus, a loss that was much greater than what would be observed after similarly located lateral lesions, including even those cases having a possible slight direct lesion of the lateral wing, but without destruction of the mediobasal tissue under the ventricular system. This marked degeneration might



9 a. Transverse section at the level of the paraventricular nuclei.



9 b. Transverse section in a plane located behind the paraventricular nuclei and anteriorly to the median eminence.

Fig. 9 a–b. Normal appearance of the paraventricular nuclei in Rat HL 439, which has fairly large periventricular lesions located behind these nuclei. Gallocyanin stain. × 25.

Table 6. *Number of magnocellular cells in the paraventricular nucleus after lesions located laterally to the nucleus.*

Rat No.	Sex	Days after op	Body wt g	Number of cells
				Paraventricular nucleus
HL 392	♂	358	270	840
451	♂	306	250	1100
456	♂	307	310	1752
472	♂	295	260	1012 r. side
472	♂	295	260	1296 l. side
255	♀	69	240	844
353	♀	561	130	904
357	♀	573	230	1248
433	♀	171	175	1196 r. side
433	♀	171	175	1080 l. side
438	♀	71	250	1496
441	♀	284	215	1372
Mean ± S.E.				1178 ± 79

Table 7. *Number of magnocellular cells in the paraventricular nuclei after lesions located immediately behind the nuclei.*

Rat No.	Sex	Days after op	Body wt g	Number of cells	
				Right paraventricular nucleus	Left paraventricular nucleus
HL 446	♂	283	275	1288	1508
437	♀	244	250	1576	1336
439	♀	262	225	1444	1156
440	♀	269	215	1052	1128
Mean ± S.E.				1340 ± 113	1282 ± 88

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Right paraventricular nucleus	10 ± 97	14	>0.1
Left paraventricular nucleus	68 ± 89	14	>0.1

therefore be ascribed, at least in part, to a retrograde degeneration of cells by interruption of nerve fibres from the paraventricular nucleus in their mediobasally situated course.

6. In sections impregnated with silver by the technique of BODIAN (1936) it was not possible to identify or trace the course of the nerve fibres from the paraventricular nucleus with any degree of certainty. Attempts were also made to count the number of axons in the infundibular stem of intact animals and of animals with complete destruction of the paraventricular nuclei in order to reveal any decrease in their number after such lesions. This, however, proved impracticable because of the small size of the numerous unmyelinated nerve fibres together with the clustering of the fibres, which all made it difficult to distinguish the individual axons.

Silver impregnation according to BODIAN after fixation in Carnoy's solution gives a very good picture of the nerve fibres. It was, however, impossible, at least in the rat, continuously to follow the course of the fine unmyelinated nerve fibres in the hypothalamus, or to determine with certainty from which nuclei or nuclear areas these fibres originated. Therefore, any attempt to trace the pathways of the various fibre systems in the hypothalamus solely on the basis of an anatomic study of normal material after silver impregnation, will rest on an insecure foundation.

DETERMINATION OF THE TRANSVERSE SECTIONAL AREA OF THE INFUNDIBULAR STEM, AND OF THE VOLUME OF THE INFUNDIBULAR PROCESS AND INTERMEDIATE LOBE AFTER COMPLETE DESTRUCTION OF THE PARAVENTRICULAR NUCLEI

Since the nerve fibres from the paraventricular nuclei pass through the infundibular stem and out into the infundibular process, it was considered of interest to carry out a quantitative analysis of the infundibular stem and process in animals with complete destruction of these nuclei.

The hypophysis of the rat grows in proportion to body weight (DONALDSON, 1915), and the rate of this growth is about the same for both the anterior and posterior lobes (MAREN & BODIAN, 1951).

In the determination of the volume of the infundibular process and intermediate lobe, and probably also in the determination of the transverse sectional area of the infundibular stem, it is presumably of great importance for the control and experimental animals to be of approximately equal body weight. That this was so in the present investigation is apparent from the accompanying tables.

After complete destruction of the paraventricular nuclei a moderate degree of atrophy of the infundibular stem was observed on qualitative examination. The atrophy was not followed by any regular and obvious microscopic changes. Thus, the hypercellularity, which, owing to the more pronounced atrophy, is a characteristic finding in the entire neurohypophysis after interruption, in front of the median eminence, of the supraoptico-hypophysial tract (FISHER, INGRAM & RANSON, 1938), could not be demonstrated after bilateral destruction of the paraventricular nuclei.

In order to form an opinion of the degree of this atrophy, quantitative measurements were made of the transverse sectional area of the infundibular stem.

The control material consisted of 11 animals, 5 males and 6 females. The body weight varied between 240 and 270 g (mean 250 ± 4.9 g) for the males, and between 200 and 250 g (mean 220 ± 7.1 g) for the females. The experimental material consisted of 15 animals, 11 males and 4 females. The body weight varied between 210 and 345 g (mean 270 ± 13.3 g) for the males, and between 200 and 270 g (mean 235 ± 14.6 g) for the females.

In the determination of any atrophy of the infundibular stem after destruction of the paraventricular nuclei it is, of course, of importance that the lesions do not involve the nerve fibres running from the supraoptic nuclei to the neurohypophysis, or, at least, that they do so as little as possible, so that any subsequent atrophy cannot be ascribed to a degeneration of these latter nerve fibres. The determination of the number of cells had shown, as mentioned above, that lesions completely destroying the paraventricular nuclei were also followed by a loss of about 20 per cent of the cells in the supraoptic nuclei.

In the controls the transverse sectional area of the infundibular

Table 8. *Transverse sectional area of the infundibular stem in intact animals.*

Rat No.	Sex	Body wt g	Transverse sectional area of the inf. stem mm²
N 152	♂	250	0.144
153	♂	250	0.130
154	♂	270	0.134
155	♂	240	0.129
156	♂	250	0.133
Mean ± S.E.		250 ± 4.9	0.134 ± 0.003
N 147	♀	220	0.154
148	♀	220	0.138
149	♀	210	0.152
150	♀	210	0.153
157	♀	250	0.146
158	♀	200	0.135
Mean ± S.E.		220 ± 7.1	0.146 ± 0.003

t-test			
Subject of the test	Difference	Degrees of freedom	P
Intact males-females	−0.012 ± 0.0044	9	<0.025

stem varied between 0.129 and 0.144 mm² (mean 0.134 ± 0.003 mm²) in the males, and between 0.135 and 0.154 mm² (mean 0.146 ± 0.003 mm²) in the females (Table 8).

In the animals with complete destruction of the paraventricular nuclei the transverse sectional area of the infundibular stem varied between 0.077 and 0.126 mm² (mean 0.103 ± 0.006 mm²) in the males, and between 0.103 and 0.122 mm² (mean 0.111 ± 0.005 mm²) in the females (Table 9).

The transverse area of the infundibular stem was, then, somewhat lower in the males than in the females, both in normal and in operated animals. A statistically significant reduction of about 20 to 25 per cent of the transverse sectional area of the infundibular stem followed the complete destruction of the paraventricular nuclei.

Table 9. *Transverse sectional area of the infundibular stem after complete destruction of the paraventricular nuclei.*

Rat No.	Sex	Days after op	Body wt g	Transverse sectional area of the inf. stem mm²
HL 270	♂	174	235	0.088
311	♂	279	300	0.077
313	♂	321	260	0.080
332	♂	347	210	0.087
336	♂	352	310	0.088
337	♂	373	345	0.123
346	♂	506	320	0.115
349	♂	573	270	0.126
363	♂	457	250	0.126
366	♂	328	210	0.099
397	♂	339	270	0.124
Mean ± S.E.			270 ± 13.3	0.103 ± 0.006
HL 405	♀	129	225	0.117
415	♀	302	270	0.122
485	♀	145	240	0.103
486	♀	151	200	0.103
Mean ± S.E.			235 ± 14.6	0.111 ± 0.005

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Males	0.031 ± 0.00925	14	< 0.005
Females	0.035 ± 0.00567	8	< 0.001

As the paraventricular nuclei contain only about 16 per cent of the total number of hypothalamic magnocellular cells, it is not remarkable that no distinct microscopic changes were observed in the infundibular stem as a consequence of the loss of the nerve fibres from these nuclei.

The material used for determining the volume of the infundibular process and intermediate lobe consisted of 7 normal animals, and of 7 animals with complete destruction of the paraventricular nuclei. All of the animals were males.

Table 10. *Volume of the infundibular process and intermediate lobe in intact animals. Fixation: Susa.*

Rat No.	Sex	Body wt g	Volume of the inf. process mm^3	Volume of the intermediate lobe mm^3
N 155	♂	240	0.71	0.43
156	♂	250	0.66	0.49
177	♂	275	0.56	0.44
178	♂	270	0.47	0.49
179	♂	295	0.65	0.37
180	♂	270	0.44	0.44
181	♂	265	0.49	0.47
Mean ± S.E.		265 ± 6.7	0.57 ± 0.04	0.45 ± 0.02

The body weight varied between 240 and 295 g (mean 265 ± 6.7 g) for the controls, and between 200 and 340 g (mean 270 ± 18.5 g) for the experimental animals. (In the experimental group the animals HL 346, 363 and 387 had received a diet containing 0.15 per cent propylthiouracil for the last 15 days before they were killed. See Section A of chapter V.)

Brolin (1948) showed that in male rats thyroidectomy produced an increase in the weight of the hypophysis owing to a significant increase in the size of both the anterior and intermediate lobes, while the absolute weight of the infundibular process remained unchanged. In female rats thyroidectomy produced no absolute or relative changes in the size of the hypophysis, neither of the whole gland, nor of its individual lobes. It is possible that the administration of propylthiouracil, which may be regarded as functional thyroidectomy, produces an analogous effect on the hypophysis.

In the normal animals the volume of the infundibular process varied between 0.44 and 0.71 mm^3 (mean 0.57 ± 0.04 mm^3), while the volume of the intermediate lobe varied between 0.37 and 0.49 mm^3 (mean 0.45 ± 0.02 mm^3) (Table 10). In the animals with complete destruction of the paraventricular nuclei the corresponding volumes varied between 0.47 and 0.65 mm^3 (mean 0.53 ± 0.03 mm^3) and between 0.26 and 0.33 mm^3 (mean 0.31 ± 0.02 mm^3), respectively (Table 11).

Table 11. *Volume of the infundibular process and intermediate lobe in animals with complete destruction of the paraventricular nuclei. Fixation: Susa.*

Rat No.	Sex	Days after op	Body wt g	Volume of the inf. process mm^3	Volume of the intermediate lobe mm^3
HL 294	♂	131	200	0.50	0.27
322	♂	172	230	0.47	0.30
331	♂	150	280	0.52	0.43
346	♂	506	320	0.64	0.30
363	♂	457	250	0.65	0.32
387	♂	281	340	0.47	0.26
389	♂	282	270	0.49	0.32
Mean $\pm$ S.E.			270 ± 18.5	0.53 ± 0.03	0.31 ± 0.02

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Infundibular process	0.04 ± 0.050	12	>0.1
Intermediate lobe	0.14 ± 0.026	12	<0.001

The volume of the infundibular process, then, did not change after complete destruction of the paraventricular nuclei, while the intermediate lobe showed a statistically significant atrophy of about one third of its normal volume.

Microscopic examination did not reveal any difference between the infundibular process of normal animals and that of animals with complete destruction of the paraventricular nuclei. The intermediate lobe, on the other hand, showed obvious changes from the normal appearance with increased density of cells owing to a decrease in the normally well developed and distinctly granular cytoplasm, in which further the granulation was no longer clearly demonstrable (Figs. 10 and 11).

DISCUSSION

It is well known that the nerve fibres from the supraoptic nuclei pass through the infundibular stem out into the infundibular

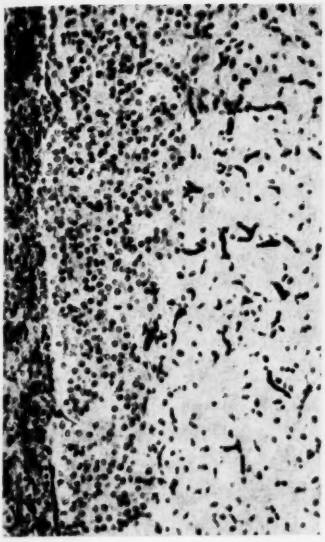


Fig. 10. The intermediate lobe of a normal animal (N 180). Azan. × 113.

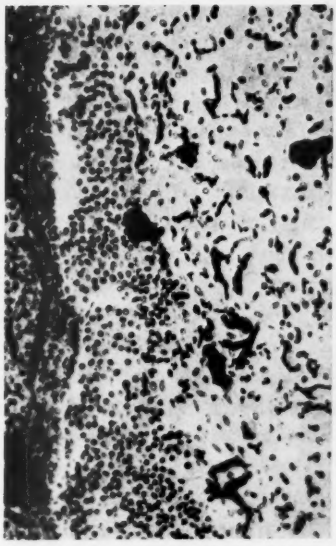


Fig. 11. The intermediate lobe of an animal with electrolytic lesions completely destroying the paraventricular nuclei (HL 322). Azan. × 113.

process. However, little is known about the course of the nerve fibres from the paraventricular nuclei, at least in the rat.

Qualitative assessment of the size of the paraventricular nucleus after transection of the hypophysial stalk or after hypophysectomy had given varying results. Thus, after hypothalamic lesions located in front of the median eminence and causing interruption of the nerve fibres from the supraoptic nuclei, FISHER, INGRAM & RANSON (1938) sometimes also observed a slight reduction in the number of cells in the paraventricular nuclei. MAGOUN & RANSON (1939), working on the monkey, estimated the cell loss in the paraventricular nuclei after transection of the hypophysial stalk to about 20 per cent of the normal number of cells, while RASMUSSEN (1940) in the hypophysectomized rat failed to demonstrate any regular occurrence of atrophy of these nuclei.

Heinbecker & White (1941) reported, in the dog, a distinct reduction in the number of cells in the paraventricular nuclei following transection of the hypophysial stalk.

However, it was not until quantitative analyses were made of the reaction of the paraventricular nuclei to such operations that more reliable information became available. Thus Frykman (1942), after hypophysectomy in the rat, found a cell loss in the paraventricular nuclei of about 35 per cent. Cell counts, performed on the dog by Pickford & Ritchie (1945), showed a marked atrophy of the paraventricular nuclei with loss of about 85 per cent of their cells in response to hypophysectomy or transection of the hypophysial stalk. O'Connor (1947) found, also in dogs, these two types of operations to result in a reduction in the number of magnocellular cells in the nuclei of about 70 and 80 per cent, respectively. Bodian & Maren (1951) carried out a quantitative analysis of the supraoptic and paraventricular nuclei after removal of about 50 per cent of the neurohypophysis along with a varying portion of the adenohypophysis. They stressed the importance of the pars anterior for preservation of part of the cells in the magnocellular hypothalamic nuclei after such operations. They thus found that if the major portion of the pars anterior was left intact, about 37 per cent of the cells in the paraventricular nuclei survived, as against only about 15 per cent after complete adenohypophysectomy with a similar neurohypophysial remnant. The corresponding figures for the supraoptic nuclei were 34 per cent and 24 per cent, respectively. They regarded these findings as evidence of the anterior lobe exerting a trophic effect on the magnocellular hypothalamic nuclei. Thus, after such partial ablation of the posterior pituitary gland Bodian & Maren noted a considerable cell loss in the paraventricular nuclei, and they assumed that practically all of the magnocellular cells in these nuclei were connected with some part of the neurohypophysis.

In accordance with these earlier investigations was the finding in the present study of the effect of interruption of the nerve fibres in the infundibular stem on the paraventricular nuclei, in which this operation was followed by a loss of about 75 per cent of their magnocellular cells.

The majority of the nerve fibres from the paraventricular nuclei thus appear to run to the infundibular process, but the loss of cells in the nuclei after transection of the hypophysial stalk is not in itself proof that the nerve fibres actually proceed through the infundibular stem. This because of the possibility that the nerve fibres from the paraventricular nuclei might terminate in relation to the supraoptic nuclei, and that an interruption of the supraopticoneurohypophysial tract is followed by transneuronal degeneration of the magnocellular cells in the paraventricular nuclei. This could, however, be excluded, because localized lesions causing marked destruction of the supraoptic nucleus did not produce any reduction in the number of magnocellular cells in the paraventricular nucleus. The infundibular stem is built up mainly of unmyelinated nerve fibres and of neuroglia. The ratio of these two types of tissue has not been determined, but probably the nerve fibres represent the major part. After complete destruction of the paraventricular nuclei the infundibular stem underwent atrophy with a reduction in the size of its transverse sectional area by 20 to 25 per cent. This atrophy cannot be ascribed entirely to the simultaneous, approximately 20 per cent reduction of the supraopticoneurohypophysial fibre system, associated with the lesions, but must probably be due mainly to the loss of nerve fibres from the paraventricular nuclei. Taken together, then, these findings suggest that the loss of cells in the paraventricular nuclei following transection of the neural stalk is a manifestation of a real retrograde degeneration, and that thus the nerve fibres from these nuclei pass through the infundibular stem.

In the rat the intrahypothalamic course of the nerve fibres extending from the paraventricular nuclei is not known with certainty.

By means of silver impregnation, a fibre tract has been demonstrated extending from the paraventricular nucleus in a ventrolateral direction, the tractus paraventricularis cinereus of Greving (1928), the lateral paraventriculo-hypophysial tract of Laruelle (1934), the tangentio-paraventricular fascicle of Roussy & Mosinger (1935). Since the individual nerve fibres could not be traced along their entire course, it was not possible, however, to

decide whether this fibre tract, which ran towards the supraoptic nucleus, terminated in relation to this nucleus or whether it joined the supraopticohypophysial fibre system.

Using GOMORI's chrome alum haematoxylin method for staining neurosecretory material, LAQUEUR (1954) demonstrated three systems of nerve fibres in the dog, extending from the paraventricular nucleus. The largest of these extended from the entire nucleus and had a course corresponding to the above-mentioned tract shown by silver impregnation to run in a ventrolateral direction towards the supraoptic nucleus. The other two fibre systems originated in the dorsocaudal tip of the paraventricular nucleus. One of these extended into the lateral hypothalamic area, reached the medial border of the internal capsule, and then bent medially so as to join the supraopticohypophysial tract after passing dorsally to the supraoptic nucleus. The other fibre system from the dorsocaudal portion of the paraventricular nucleus represented the shortest and at the same time a direct connection between this nucleus and the neurohypophysis, the nerve fibres looping over the fornix, then swerving medially so as to enter the infundibular stem caudally to the supraopticohypophysial tract. In the region where the course of the nerve fibres belonging to the supraoptic nucleus approaches that of the paraventricular nucleus it is not possible to distinguish the two fibre systems by Gomori-staining. LAQUEUR was therefore unable to decide with certainty whether nerve fibres extending from the paraventricular nucleus and passing in the vicinity of the supraoptic nucleus, also terminated in relation to the latter.

In the present work the course of the nerve fibres from the paraventricular nucleus was studied by the indirect method involving a quantitative analysis of the nucleus after electrolytic lesions in various regions of the hypothalamus. Such an analysis should yield some information on the pathway available for the nerve fibres from the paraventricular nucleus.

Since, as mentioned before, a pronounced destruction of the supraoptic nucleus produced no reduction in the number of magnocellular cells in the paraventricular nucleus, it is less likely that the nerve fibres from the latter terminate in relation to the supraoptic nucleus or pass in its immediate vicinity.

However, in a pituitary dwarf with severe, permanent diabetes insipidus Baker & Craft (1940) observed basal changes with vacuolization and glial proliferation completely destroying the supraoptic nucleus on both sides, and in this patient the paraventricular nuclei, without being directly involved in the lesions, showed a practically total loss of cells. They interpreted this finding as a retrograde degeneration and concluded that the main part of the nerve fibres from the paraventricular nuclei ended in relation to the supraoptic nuclei. However, the pronounced basal destruction also involved the median eminence, so that the marked loss of cells in the paraventricular nuclei must instead be conceived as due to interruption in the median eminence of the hypothalamoneurohypophysial fibre system, containing nerve fibres from both the supraoptic and paraventricular nuclei. In this case the effect was therefore more like that of transection of the hypophysial stalk, an assumption further supported by the infantile status of the patient.

If the major part of the nerve fibres from the paraventricular nucleus extend in a lateral direction, hypothalamic lesions situated immediately laterally to the nucleus should cause a retrograde degeneration of its cells. The results of the determination of the number of cells in the paraventricular nucleus after laterally placed lesions leaving only a narrow band of intact tissue immediately outside the magnocellular portion made it probable, however, that the main part of the nerve fibres from the nucleus do not proceed in a lateral direction, at least not for any longer distance.

Electrolytic lesions placed behind the paraventricular nucleus further showed that the nerve fibres cannot extend from this nucleus in a posterior direction along with and parallel to the ventricular wall or along the base of the brain immediately outside the median eminence.

Discrete lesions placed below the paraventricular nucleus and lying close to the wall of the third ventricle produced no reduction in the number of magnocellular cells in the nucleus. Thus, the nerve fibres from the paraventricular nucleus do not seem to pass close to the ventricular ependyma straight down towards the base of the brain.

Experiments with transection of the hypophysial stalk have shown that at least a great part of the nerve fibres from the paraventricular nucleus pass through the infundibular stem. It is therefore not likely that they extend in a dorsal direction. Nor were lesions placed above the paraventricular nucleus followed by any demonstrable signs of retrograde degeneration.

Fairly large lesions in the vicinity of the paraventricular nucleus thus showed that at least the major portion of the nerve fibres from this nucleus does not seem to extend in a ventrolateral, caudal, dorsal, or directly lateral direction. *The route left for the nerve fibres from the paraventricular nucleus, therefore, appears to lie along a pathway first swerving gently outwards, and then bending sharply downwards in a basocaudal direction so as to occupy, just in front of the median eminence, a mediobasal position under the floor of the third ventricle.* A further support for the assumption that the nerve fibres from the paraventricular nucleus soon reach a medial position, is provided by the fact that a greater cell loss was obtained in the paraventricular nucleus after laterally placed lesions partially involving also the mediobasal tissue in front of the median eminence, than after similarly situated lesions sparing the latter structure.

In front of the median eminence the fibre tracts from the two magnocellular hypothalamic nuclei will thus lie close to each other under the floor of the third ventricle. After lesions causing an interruption of the nerve fibres or a destruction of the nuclei of origin of one of these two main fibre systems from the anterior hypothalamus to the neurohypophysis, it is also of great importance – which has earlier never been stressed – to determine the extent to which nerve fibres belonging to the other fibre system have been involved in the lesions. Thus, in the present study, the supraoptic nuclei, as judged by microscopic inspection, appeared normal in animals with complete destruction of the paraventricular nuclei, and they would qualitatively also have been classified as such. *The quantitative analysis showed, however, that localized lesions resulting in complete destruction of the paraventricular nuclei also caused a decrease in the size of the supraopticoneurohypophysial fibre system with a loss of about 20 per cent of the cells in the supraoptic nuclei.* This finding thus

shows the value of performing a quantitative analysis of the reaction of the cells in adjacent nuclei or nuclear areas for a full evaluation of the effect of a certain type of hypothalamic lesions. The cell reduction occurring in the supraoptic nuclei after complete destruction of the paraventricular nuclei might possibly be due to the lesions encroaching upon the dorsal part of the supraopticoneurohypophysial fibre tract. Since, however, the lesions in their entire extent were situated close to the ventricular system medially to a plane through the lateral margin of the optic chiasma, and since in the frontal plane they started above the posterior end of the supraoptic nuclei to terminate in the form of narrow tissue necroses above the anterior end of the median eminence without reaching the base of the brain, it seems hardly probable that they could involve the supraopticoneurohypophysial tract to any greater extent. Another possible explanation of the cell loss in the supraoptic nuclei following complete destruction of the paraventricular nuclei, might be that the major portion of the cells remaining after transection of the hypophysial stalk, about 25 per cent of the total number of cells, send their axons not towards the infundibular stem but to the paraventricular nuclei. They will therefore, after destruction of the nuclei, disappear because of retrograde degeneration. This material, however, does not admit of any conclusions concerning the validity of such an assumption. However, since the examination of the extent of these lesions appeared to exclude any direct involvement of the supraoptic nuclei or their fibre tracts running to the neurohypophysis, this interpretation seems to be the most likely for the time being. *There is thus a possibility that about 20 per cent of the cells in the supraoptic nuclei send their axons to the paraventricular nuclei.* This possibility must be borne in mind in experimental work on the hypothalamoneurohypophysial system. The significance of such a connection between the two magnocellular hypothalamic nuclei cannot at present be evaluated.

Interruption of the supraopticohypophysial fibre tract in front of the median eminence is followed by a marked atrophy of the infundibular process (Fisher, Ingram & Ranson, 1938). After complete destruction of the paraventricular nuclei, on the other

hand, no reduction in the volume of the infundibular process was found in the present investigation.

Atrophy of the infundibular process in response to various operations on the hypothalamoneurohypophysial system might possibly be due to loss of trophic influence, or to a real loss of nervous tissue because of a degeneration of nerve fibres. If atrophy due to loss of trophic influence on the infundibular process should require practically complete denervation of the organ, no atrophy would be expected after destruction of the paraventricular nuclei. If, on the other hand, atrophy of the infundibular process should be due to loss of tissue, it is likewise probable that the part of the total volume of the infundibular process occupied by the nerve fibres from the paraventricular nuclei would be too small for a loss of these to produce any demonstrable atrophy. In addition, should the nerve fibres from the supraoptic nuclei be more richly arborized than the nerve fibres from the paraventricular nuclei, the volume of these latter would constitute a still smaller percentage of the total volume of the infundibular process.

The paraventricular nuclei contain about 16 per cent of the total number of magnocellular cells in the hypothalamus, but since lesions destroying these nuclei also produced a reduction of the cells in the supraoptic nuclei, the total loss of magnocellular cells was about 35 per cent. A decrease in the number of nerve cells from the two magnocellular hypothalamic nuclei by about one third was thus not large enough to produce a measurable atrophy of the infundibular process. As shown by Fisher, Ingram & Ranson (1938) the interruption, in front of the median eminence, of the nerve fibres to the neurohypophysis will be followed by atrophy of the infundibular process, which might be expected especially as such lesions will involve the greater part of the axons of the hypothalamic magnocellular cells. It is less likely that this effect would follow a localized destruction of the paraventricular nuclei with slight involvement also of the supraopticoneurohypophysial fibre system. The degree of degeneration of the hypothalamoneurohypophysial system after complete destruction of the paraventricular nuclei would thus not be sufficient to cause atrophy of the infundibular process.

On the other hand, since the major portion of the infundibular stem is probably built up of nerve fibres extending from the hypothalamus to the posterior pituitary gland, the loss of the fibre system from the paraventricular nuclei may explain the atrophy of the transverse sectional area of the infundibular stem that occurred after complete destruction of the nuclei.

The intermediate lobe receives nerve fibres from the infundibular process, probably coming from the hypothalamoneurohypophysial fibre system, as shown among others by CAJAL (1894), TELLO (1912), HAIR (1937, 1938), HILLARP & JACOBSOHN (1943), TRUSCOTT (1944), HILLARP (1946), GREEN & HARRIS (1947), GREEN (1951).

HILLARP & JACOBSOHN (1943) using a methylene blue staining technique (HILLARP, 1946), showed that in the rat interruption of the hypothalamoneurohypophysial fibre system, either by extirpation of the posterior pituitary gland or by transection of the hypophysial stalk, caused a disappearance of the nerve fibres in the intermediate lobe. In the present study lesions causing complete destruction of the paraventricular nuclei, including also the attendant about 20 per cent reduction of the supraopticoneurohypophysial fibre system, were followed by a decrease of the volume of the intermediate lobe by about one third. It is possible that this decrease in volume might be a consequence of a denervation of the intermediate lobe following the destruction of the paraventricular nuclei. No determination was made of the amount of intermedin in the atrophied intermediate lobe. In contrast to this finding, BOGDANOVE & HALMI (1953) and BOGDANOVE, SPIRTOS & HALMI (1955) reported an apparent hyperplasia of the intermediate lobe after hypothalamic lesions, but they did not give any more detailed description of the position and extent of their lesions, nor did they make any quantitative determination of the volume of the intermediate lobe.

The parvocellular portion of the paraventricular nucleus was not studied, because being difficult to delimit, it does not lend itself to a more exact quantitative analysis. However, no appreciable cell loss was observed in the parvocellular portion after transection of the hypophysial stalk. Therefore, it appears less likely that these nerve cells are to any greater extent in direct connection with the infundibular process.

SUMMARY

The reaction of the hypothalamoneurohypophysial fibre system to transection of the hypophysial stalk and to electrolytic lesions in various regions of the hypothalamus was studied by quantitative morphologic analyses.

1. Transection of the hypophysial stalk produced a reduction in the number of magnocellular cells in the paraventricular nuclei by about 75 per cent. Since localized, marked destruction of the supraoptic nuclei did not cause any cell loss in the paraventricular nuclei, the reduction seen after transection of the hypophysial stalk could not be ascribed to a transneuronal degeneration following interruption of the hypothalamoneurohypophysial fibre system. The major part of the nerve fibres from the magnocellular portion of the paraventricular nuclei thus appears to pass through the infundibular stem. Further support for this assumption was provided by the reduction observed in the transverse sectional area of the infundibular stem of about 20 to 25 per cent, occurring after complete destruction of the paraventricular nuclei, which might be regarded as a result of a loss of nerve fibres.

2. Analysis of the extension of electrolytic lesions placed in various regions of the hypothalamus, together with a determination of the number of magnocellular cells in the paraventricular nucleus, made it probable that the nerve fibres from the magnocellular portion of this nucleus swerve gently outwards, then bend sharply downwards in a basocaudal direction and, just in front of the median eminence, occupy a mediobasal position under the floor of the third ventricle.

The magnocellular cells of the paraventricular nucleus do not to any greater extent send their axons to the supraoptic nucleus, since marked destruction of the latter was not followed by any signs of cell loss in the paraventricular nucleus.

3. Lesions causing complete destruction of the paraventricular nuclei, including an about 20 per cent reduction of the size of the supraopticoneurohypophysial fibre system, did not quantitatively affect the volume of the infundibular process, but produced a significant decrease of about one third in the volume of the intermediate lobe.

4. Localized, complete destruction of the paraventricular nuclei resulted in a reduction of the number of magnocellular cells in the supraoptic nuclei by about 20 per cent. Since no direct destruction of the supraoptic nuclei or of their fibre tracts to the neurohypophysis could be demonstrated, it seems possible that about 20 per cent of the cells in the supraoptic nuclei send their axons to the paraventricular nuclei.

5. No marked reduction in the number of cells in the parvocellular portion of the paraventricular nucleus could be demonstrated after transection of the hypophysial stalk, resulting in a loss of about 75 per cent of the magnocellular cells in the nucleus. Therefore, it does not seem probable that the parvocellular portion has a direct connection with the infundibular process to any greater extent. Since, however, a more exact determination of the number of cells in this portion of the paraventricular nucleus is difficult to perform, the possibility cannot be precluded that also some of these cells send their axons to the neurohypophysis or to the pars intermedia of the pituitary gland.

6. The decrease in volume of the intermediate lobe following destruction of the paraventricular nuclei might possibly be due to loss of innervation from the paraventricular region. It cannot be decided, however, from what portion of the paraventricular nucleus, or any adjacent nuclear area, such an innervation would be exerted.

Chapter V

THE PARAVENTRICULAR NUCLEI AND THE REGULATION OF THE SECRETION OF THE ANTERIOR PITUITARY HORMONES

It is generally accepted that the anterior pituitary gland is under neural control from the hypothalamus (HARRIS, 1948 d, 1955). Further, in the last few years the importance of the hypophysial portal circulation for the function of the anterior lobe of the pituitary gland has been stressed, and it has been shown that for normal activity the gland is dependent on its hypophysial portal blood supply.

The present, most likely hypothesis concerning the nature of such a hypothalamic control seems to be the assumption of a liberation of some humoral substance or substances into the capillaries of the primary plexus of the hypophysial portal vessels, located in the median eminence of the tuber cinereum. This substance is then held to be carried by the blood stream to the adenohypophysis, there influencing the activity of the glandular cells. A detailed account of the present theory of a neurovascular control of the activity of the anterior pituitary gland may be found in the extensive monograph by G. W. HARRIS (HARRIS, 1955).

Some earlier experiments, the results of which will be discussed in the following sections, provided some grounds for suspecting the paraventricular region to be concerned with the regulation of the secretion of some of the anterior pituitary hormones. In order to form a clearer opinion as to whether such a control does exist, a study was made of the secretion of the thyrotrophic, adrenocorticotrophic and gonadotrophic hormones in animals with hypothalamic lesions completely or partially destroying the paraventricular nuclei.

A. THE PARAVENTRICULAR NUCLEI AND THE REGULATION OF THE SECRETION OF THYROTROPHIC HORMONE

The intact connections between the hypothalamus and the hypophysis seem to be essential for the secretion of the thyrotrophic hormone, at least under conditions making higher demands on thyrotrophic function (UOTILA, 1939 a and b, BROLIN, 1945, 1947, BOGDANOVE, SPIRTOS & HALMI, 1955). Further WESTMAN & JACOBSOHN (1938) and WESTMAN, JACOBSOHN & OKKELS (1942) showed, in rats and in rabbits respectively, that transection of the hypophysial stalk was sometimes followed by microscopic signs of diminished thyroid activity.

GREER (1951, 1952) made electrolytic lesions in the rat in an attempt to determine whether the secretion of thyrotrophic hormone by the adenohypophysis is controlled by the hypothalamus. He found that symmetric lesions placed within a region extending from the suprachiasmatic to the caudal ventromedial nuclei prevented the thyroid hyperplasia normally following thiouracil feeding. However, since the iodide-concentrating capacity was almost normal in these animals, GREER (1952) assumed the occurrence of two thyrotrophic principles, one with a growth effect and one with a metabolic effect. BOGDANOVE & HALMI (1952, 1953) and BOGDANOVE, SPIRTOS & HALMI (1955) confirmed these findings, but they supposed (HALMI, SPIRTOS, BOGDANOVE & LIPNER, 1953, HALMI & SPIRTOS, 1954) that the propylthiouracil induced elevation of the thyroid/serum iodide concentration ratio was dependent on a potentiation, by thiouracil, of the iodide concentrating mechanism of the thyrotrophic hormone, which, however, was secreted in too small an amount in animals with such lesions to exert any demonstrable morphologic effect on the thyroid gland.

GANONG, FREDRICKSON & HUME (1954, 1955) observed that lesions placed in and just above the median eminence in the dog produced thyroid atrophy as well as a decrease in the uptake of radioactive iodine by the gland.

In a short communication GREER in collaboration with ERWIN (GREER & ERWIN, 1954) later suggested, on the basis of an analysis of the extension of hypothalamic lesions effective in preventing the development of thyroid hyperplasia on administration of propylthiouracil, that the region of the paraventricular nuclei is intimately concerned with the control of the secretion of thyrotrophic hormone. Further GREER (1955 a) showed that such lesions do not interfere with the response of the thyroid gland to injected thyrotrophic hormone. Thus, the inability of propylthiouracil to produce hyperplasia could not be ascribed to any irresponsiveness of the thyroid to thyrotrophin. He therefore interpreted it as an inability on the part of the adenohypophysis to increase its secretion of thyrotrophic hormone.

In the present experiments the morphologic reaction of the thyroid gland to propylthiouracil was studied in animals with hypothalamic lesions in the region of the paraventricular nuclei.

MATERIAL AND METHODS

Diet. – Those animals that were fed a goitrogenic agent received it as 0.15 per cent propylthiouracil in a modified BILLS's diet (BILLS, HONEYWELL, WIRICK & NUSSMEIER, 1931) consisting of:

Maize	58.75 per cent
Milk powder	18.75
Soya-bean oil	6.25
Linseed meal	12.50
Luzern meal	1.50
Wheat seeds	1.25
NaCl	0.50
$CaCO_3$	0.50

The animals were allowed to eat and drink *ad libitum*.

After having received this propylthiouracil-containing diet for 16 or 17 days the animals were killed.

Normal animals. – Examination was made of the thyroid from 21 untreated animals and from 12 animals that had received propylthiouracil-containing diet during the same period as the animals with hypothalamic lesions.

Animals with hypothalamic lesions. – Without previous administration of propylthiouracil-containing diet the thyroid gland was examined in 9 animals with complete destruction of the paraventricular nuclei; in 6 animals, 2 of which had lesions located laterally to and 4 lesions located immediately behind the paraventricular nuclei, in none encroaching upon these nuclei; and further in 11 animals with direct, partial destruction of the supraoptic nuclei.

The effect of propylthiouracil on the gross morphology and microscopic appearance of the thyroid was studied in 10 animals with partial or complete destruction of the paraventricular nuclei.

Histologic procedures. – The thyroid glands of the propylthiouracil treated animals were fixed in Susa for 24 hours. This fixing fluid causes less shrinkage than the commonly used fixing fluids, and further it is most suitable for cytologic study of the thyroid (Borell, 1945, Holmgren & Nilsonne, 1948). Serial sections were cut at 5 μ, each fifth section being mounted and stained with haematoxylin and eosin.

RESULTS

NORMAL ANIMALS

In the normal animals the absolute weight of the thyroid gland of the males varied between 16.9 and 42.9 mg (mean 31.7 ± 1.7 mg), the corresponding figures for the females being 23.9 and 39.1 mg (mean 29.4 ± 2.6 mg). The relative weight of the thyroid, expressed per 100 g body weight, varied between 8.0 and 17.9 mg (mean 11.8 ± 0.7 mg) for the males, and between 11.4 and 15.6 mg (mean 13.4 ± 0.8 mg) for the females (Table 12). The weight of the thyroid was not found to vary with sex.

Table 12. *Weight of the thyroid in the normal animals.*

Rat No.	Sex	Body wt g	Thyroid wt mg	Thyroid wt mg/ 100 g body wt
N 97	♂	315	31.4	10.0
98	♂	275	31.1	11.3
99	♂	320	30.2	9.4
100	♂	210	16.9	8.0
101	♂	300	25.6	8.5
102	♂	250	29.8	11.9
103	♂	290	34.6	11.9
104	♂	250	30.9	12.4
105	♂	285	31.6	11.1
106	♂	280	25.5	9.1
151	♂	285	40.3	14.1
152	♂	250	24.0	9.6
153	♂	250	37.7	15.1
154	♂	240	38.4	14.2
155	♂	240	42.9	17.9
156	♂	250	36.0	14.4
Mean ± S.E.		270 ± 7.6	31.7 ± 1.7	11.8 ± 0.7
N 148	♀	220	26.0	11.8
149	♀	210	23.9	11.4
150	♀	210	29.0	13.8
157	♀	250	39.1	15.6
158	♀	200	29.2	14.6
Mean ± S.E.		220 ± 8.6	29.4 ± 2.6	13.4 ± 0.8

t-test			
Subject of the test	Difference	Degrees of freedom	P
Thyroid wt mg males-females	2.3 ± 3.34	19	>0.1
Thyroid wt mg/100 g body wt males-females	—1.6 ± 1.32	19	>0.1

OPERATED ANIMALS NOT FED WITH PROPYLTHIOURACIL

All animals with lesions in the region of the supraoptic nuclei were males. The absolute weight of the thyroid gland varied between 22.0 and 49.4 mg (mean 37.0 ± 3.5 mg), while the corresponding relative values were 7.6 and 18.2 mg (mean 12.4 ± 0.9 mg) (Table 13).

Table 13. *Weight of the thyroid in animals with direct, partial destruction of the supraoptic nuclei.*

Rat No.	Sex	Days after op	Body wt g	Thyroid wt mg	Thyroid wt mg/ 100 g body wt
HL 448	♂	307	290	22.0	7.6
449	♂	308	395	49.4	12.5
461	♂	106	300	46.5	15.5
462	♂	176	185	22.0	11.9
464	♂	99	290	43.5	15.0
469	♂	99	265	48.3	18.2
471	♂	303	370	46.1	12.5
474	♂	295	235	23.1	9.8
475	♂	295	380	43.0	11.3
476	♂	297	345	39.4	11.4
478	♂	297	220	23.9	10.9
Mean ± S.E.			300 ± 20.8	37.0 ± 3.5	12.4 ± 0.9

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Thyroid wt mg	−5.3 ± 3.52	25	>0.1
Thyroid wt mg/100 g body wt	−0.6 ± 1.10	25	>0.1

The animals with lesions located either laterally to or behind the paraventricular nuclei were all females. In this group the absolute weight of the thyroid varied between 21.0 and 45.2 mg (mean 32.1 ± 3.7 mg). The relative weight of the gland ranged from 11.4 to 20.1 mg (mean 14.8 ± 1.3 mg) (Table 14).

Thus, neither the lesions located in the region of the supraoptic nuclei, nor those located laterally to or behind the paraventricular nuclei, were followed by any thyroid atrophy.

Likewise, there was no significant change in the weight of the thyroid after complete destruction of the paraventricular nuclei. The absolute weight of the gland thus varied between 24.7 and 49.2 mg (mean 35.1 ± 4.5 mg) for the males and between 23.5 and 36.6 mg (mean 27.8 ± 3.0 mg) for the females. The corresponding relative weights varied between 9.0 and 18.2 mg (mean 13.3 ± 1.7 mg) and between 8.7 and 17.7 mg (mean 13.3 ± 2.2 mg) (Table 15).

Table 14. *Weight of the thyroid in animals with lesions located laterally to or behind the paraventricular nuclei.*

Rat No.	Sex	Days after op	Body wt g	Thyroid wt mg	Thyroid wt mg/100 g body wt	Location of the lesions
HL 441	♀	284	215	34.5	16.0	Lateral to the nuc
484	♀	147	200	23.4	11.7	Lateral to the nuc
437	♀	249	250	37.1	14.8	Behind the nuclei
439	♀	267	225	45.2	20.1	Behind the nuclei
440	♀	274	215	31.3	14.6	Behind the nuclei
499	♀	142	185	21.0	11.4	Behind the nuclei
Mean ± S.E.			215 ± 9.0	32.1 ± 3.7	14.8 ± 1.3	

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Thyroid wt mg	—2.7 ± 4.68	9	>0.1
Thyroid wt mg/100 g body wt	—1.4 ± 1.61	9	>0.1

PROPYLTHIOURACIL FED ANIMALS

Feeding with propylthiouracil produced an increase in the weight of the thyroid in normal animals as well as in animals with partial or complete destruction of the paraventricular nuclei.

Both the controls and the experimental animals were males.

In the intact animals the absolute weight of the thyroid varied between 39.7 and 105.0 mg (mean 66.7 ± 5.9 mg), while the relative weight of the gland ranged from 16.6 to 39.1 mg (mean 26.1 ± 2.2 mg) (Table 16). The absolute weight of the thyroid of the animals with electrolytic lesions located in the region of the paraventricular nuclei varied between 41.7 and 121.5 mg (mean 73.6 ± 7.7 mg), the corresponding relative values being 17.7 and 34.2 mg (mean 25.3 ± 1.8 mg) (Table 17).

Thus, judging by the pronounced growth of the thyroid, discrete lesions completely or partially destroying the paraventricular nuclei did not prevent the increased secretion of thyrotrophic hormone from the adenohypophysis in response to propylthiouracil feeding.

Table 15. *Weight of the thyroid in animals with complete destruction of the paraventricular nuclei.*

Rat No.	Sex	Days after op	Body wt g	Thyroid wt mg	Thyroid wt mg/ 100 g body wt
HL 349	♂	573	270	49.2	18.2
391	♂	357	255	41.7	16.4
394	♂	361	250	31.7	12.7
397	♂	339	270	28.0	10.4
487	♂	106	275	24.7	9.0
Mean ± S.E.			265 ± 4.9	35.1 ± 4.5	13.3 ± 1.7
HL 415	♀	312	270	23.5	8.7
436	♀	247	150	26.5	17.7
479	♀	145	220	36.6	16.6
485	♀	145	240	24.6	10.3
Mean ± S.E.			220 ± 25.5	27.8 ± 3.0	13.3 ± 2.2

t-test			
Subject of the test	Difference from the intact animals	Degrees of freedom	P
Thyroid wt mg, males	−3.4 ± 3.86	19	>0.1
Thyroid wt mg/100 g body wt, males	−1.5 ± 1.55	19	>0.1
Thyroid wt mg, females	1.6 ± 3.96	7	>0.1
Thyroid wt mg/100 g body wt, females	0.1 ± 2.17	7	>0.1

After administration of propylthiouracil the thyroid glands of intact animals, as well as those of animals with complete or partial destruction of the paraventricular nuclei, showed a highly active microscopic appearance with marked increase in cell height and almost complete loss of colloid in the follicles (Figs. 12 and 13). As is apparent from Fig. 14, which shows the microscopic appearance of the thyroid of an intact, untreated rat, there is an obvious difference in degree of thyroid activity between untreated and propylthiouracil treated animals.

Microscopic examination of the anterior lobe from propylthiouracil fed animals failed to reveal any distinct differences between the control and experimental groups.

Table 16. *Weight of the thyroid in normal animals after administration of 0.15 % propylthiouracil.*

Rat No.	Sex	Body wt g	Thyroid wt mg	Thyroid wt mg/ 100 g body wt
N 76	♂	290	73.1	25.2
77	♂	290	48.0	16.6
78	♂	270	74.8	27.7
79	♂	220	86.1	39.1
80	♂	230	74.9	32.6
81	♂	280	105.0	37.5
82	♂	220	39.7	18.0
83	♂	210	39.9	19.0
84	♂	260	69.2	26.6
85	♂	250	46.3	18.5
86	♂	285	60.6	21.3
87	♂	265	82.2	31.0
Mean $\pm$ S.E.		255 $\pm$ 8.5	66.7 $\pm$ 5.9	26.1 $\pm$ 2.2

t-test			
Subject of the test	Difference from intact not propylthiouracil fed animals	Degrees of freedom	P
Thyroid wt mg	-35.0 ± 5.39	26	<0.001
Thyroid wt mg/100 g body wt	-14.3 ± 2.08	26	<0.001

DISCUSSION

The experiments clearly showed that complete or partial destruction of the paraventricular nuclei did not prevent the normal goitrogenic response to propylthiouracil feeding.

In the animals HL 388 and 389, for which the lowest absolute weights of the thyroid glands were found after the administration of propylthiouracil to operated animals, the lesions were more extensive than usual with slight involvement also of the mediobasal tissue under the floor of the third ventricle. Microscopically, however, both these glands showed a highly active picture.

Lesions markedly destroying the mediobasal tissue in front of and in the anterior end of the median eminence will probably cause an interruption of most of the nerve fibres passing from

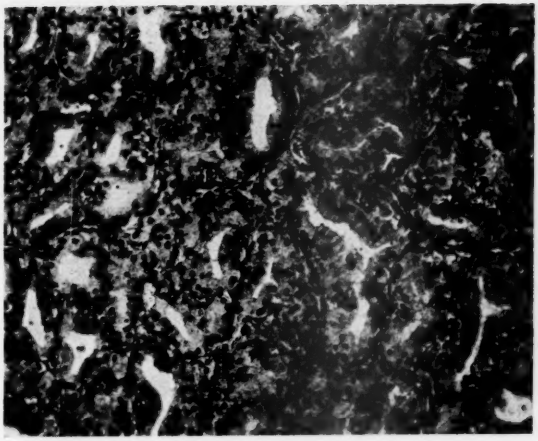


Fig. 12. Thyroid hyperplasia in a normal animal fed on a diet containing propylthiouracil (N 81). Haematoxylin–eosin. × 113.

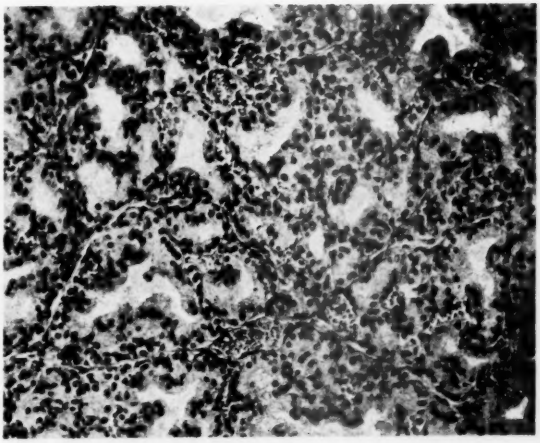


Fig. 13. Thyroid hyperplasia in an animal with electrolytic lesions completely destroying the paraventricular nuclei, and fed on a diet containing propylthiouracil (HL 346). Haematoxylin–eosin. × 113.

Table 17. *Weight of the thyroid in animals with complete or partial destruction of the paraventricular nuclei after administration of 0.15% propylthiouracil.*

Rat No.	Sex	Days after op	Body wt g	Thyroid wt mg	Thyroid wt mg/100 g body wt	Number of remaining cells in the combined paraventr. nuclei
HL 346	♂	506	320	103.5	32.3	0
363	♂	457	250	69.1	27.6	0
387	♂	281	340	68.3	20.1	0
388	♂	282	205	41.7	20.3	0
389	♂	282	270	47.9	17.7	0
365	♂	457	355	121.5	34.2	880
401	♂	276	295	57.5	19.5	948
360	♂	457	345	86.2	25.0	1232
344	♂	506	230	69.4	30.2	1256
345	♂	506	270	71.0	26.3	1956
Mean ± S.E.			290 ± 16.3	73.6 ± 7.7	25.3 ± 1.8	

t-test			
Subject of the test	Difference from intact propylthiouracil fed animals	Degrees of freedom	P
Thyroid wt, mg	−6.9 ± 9.52	20	> 0.1
Thyroid wt mg/100 g body wt	0.8 ± 2.96	20	> 0.1

the anterior hypothalamus to the pituitary gland, and might therefore produce an effect almost comparable to that of section of the hypophysial stalk. This is of importance since BROLIN (1945) showed that transection of the pituitary stalk in the rat was followed by a diminished thyroid activity because of a decreased thyrotrophic function. It should therefore be stressed that in using hypothalamic lesions for the localization of an area or a nucleus controlling the secretion of a hypophysial hormone, it is necessary to avoid, as far as possible, any destruction of the mediobasal tissue immediately in front of and in the anterior end of the median eminence. The more this mediobasal region is destroyed, the more the effect of the lesions will resemble that seen after transection of the hypophysial stalk, with the result

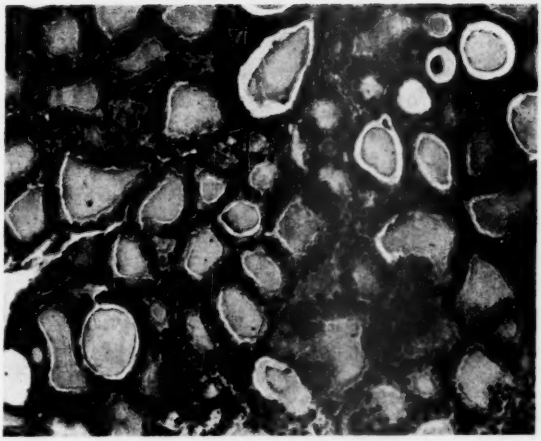


Fig. 14. Thyroid gland of a normal animal not given any goitrogenic diet (N 102). Haematoxylin–eosin. × 113.

that it will no longer be possible to analyze the significance of different nuclear areas for specific hypophysial functions.

However, this does not seem to have received due consideration in earlier investigations, and the lack of thyroid hyperplasia in response to propylthiouracil feeding in the experiments of GREER (1951, 1952), BOGDANOVE & HALMI (1953), and GREER & ERWIN (1954) might, as far as can be judged from the rather brief descriptions of the extension of their lesions, be due to a destruction, in the region of the median eminence, of the nervous link in the neurovascular hypothalamohypophysial connections. Since BOGDANOVE & HALMI reported that the effective lesions, which were situated basally near the midline and extending from the optic chiasma to the anterior end of the median eminence, also caused an atrophy of the posterior pituitary gland, this further supports the assumption that there was actually an interruption of most of the nerve fibres of the hypothalamoneurohypophysial tract.

Analogously, the finding of GANONG, FREDRICKSON & HUME (1954, 1955) that lesions in and just above the anterior end of the

median eminence were followed by thyroid atrophy as well as by marked decrease in the uptake of radioactive iodine by the gland, shows the importance of an intact connection between the hypothalamus and the hypophysis, but it does not provide any clear evidence of the location of a hypothalamic area controlling the secretion of thyrotrophic hormone.

By using discrete lesions not involving the mediobasal tissue in front of and in the median eminence, it was shown in the present experiments that the paraventricular nuclei probably do not participate in the hypothalamic control of the secretion of thyrotrophic hormone from the anterior pituitary gland.

SUMMARY

Lesions completely or partially destroying the paraventricular nuclei did not prevent the goitrogenic response to propylthiouracil feeding. Thus, these nuclei do not seem to be concerned with the control of the secretion of thyrotrophic hormone from the anterior pituitary gland.

B. THE PARAVENTRICULAR NUCLEI AND THE REGULATION OF THE SECRETION OF ADRENOCORTICOTROPHIC HORMONE

It has been made highly probable that the hypothalamus is of essential importance for the transmission of the stimulus of stress to the pituitary gland.

Thus, De Groot & Harris (1950, 1952) found that stimulation of the posterior region of the tuber cinereum or the mammillary body resulted in a lymphopenia similar to that following emotional stress stimulus or intravenous injection of adrenocorticotrophic hormone (ACTH), while electrolytic lesions in these regions or in the zona tuberalis at the anterior pole of the pituitary gland reduced or abolished the lymphopenic response to emotional stress. On the basis of their experiments they inferred the existence of a neurohumoral control of adrenocorticotrophic secretion from the anterior pituitary gland, the vascular link being represented by the hypophysial portal vessels. They thus assumed the electrolytic lesions to cause an interruption of the neurovascular hypothalamo-hypophysial connections either by destruction of the nerve fibres running to the primary plexus of the hypophysial portal vessels in the median eminence, or by direct destruction of these portal vessels in the zona tuberalis. Electric stimulation, on the other hand, was followed by signs of discharge of ACTH only when applied to those posterior regions mentioned, while stimulation in the zona tuberalis, where the effective structure consists of the hypophysial portal vessels, was without effect.

Hume & Wittenstein (1950), working on the dog, found that paramedian lesions in the anterior hypothalamus and at the juncture of the middle and posterior hypothalamus prevented the eosinopenic response to stress. In later publications Hume (1952, 1953) reported that also lesions located in the anterior end of the

median eminence or in the posterior tuber cinereum and involving the anterior portion of the mammillary body blocked the acute release of ACTH from the adenohypophysis, while stimulation of the median eminence or of the posterior tuber cinereum resulted in eosinopenia. These findings thus agreed essentially with those of De Groot & Harris.

Porter (1952, 1953, 1954) found that under stressing conditions an increased electric activity from the tuberal and mammillary regions could be recorded in the cat and monkey, and further that stimulation of the posterior tuber cinereum or the mammillary body was followed by eosinopenia, while electrolytic destruction of these regions prevented the eosinopenic response to stressing agents.

McCann (1953) and Laqueur, McCann, Schreiner, Rosemberg, Rioch & Anderson (1955), working on the rat and cat, respectively, found that destruction of the median eminence markedly reduced or prevented the adrenal cortical response to stressful stimuli. Similar results were also reported by Anand, Raghunath, Dua & Mohindra (1954). They demonstrated that hypothalamic lesions in the rat, located in the medial part of the anterior hypothalamus in a plane corresponding to the position of the paraventricular nuclei – without, however, directly involving these structures – and in the anterior end of the median eminence, in the plane of the ventromedial nuclei, prevented the eosinopenic response to subcutaneously injected hypertonic saline, but not to adrenaline. After stimulation of the anteromedial portion of the median eminence in the cat, Anand & Dua (1955) obtained a reduction of 25 per cent or more of the number of circulating eosinophils, a finding further supporting their earlier assumption that this region is of importance for the secretion of ACTH from the anterior pituitary gland. Destruction of the median eminence in the dog has been shown to prevent the adrenal cortical hypertrophy normally following repeated trauma or unilateral adrenalectomy (Ganong & Hume, 1954).

The purpose of the present experiments was to determine whether an intact paraventriculoneurohypophysial fibre system is necessary for the acute release of ACTH from the anterior pituitary gland in response to stress.

METHODS

The depletion of adrenal cortical cholesterol in response to stress was used as the index of ACTH discharge from the pituitary gland. The cholesterol content of the right adrenal was taken as the starting level, and the decrease in cholesterol concentration in the left adrenal after stress was used as the criterion for a release of ACTH.

Stressing procedure. — For determination of the cholesterol concentration before stress the right adrenal was extirpated under ether anaesthesia.

In order to prevent the secretion of adrenaline from the remaining adrenal gland, and thereby any direct effect of this hormone on the anterior pituitary gland, the left splanchnic nerve was divided. This procedure denervates the adrenal medulla in the rat (HILLARP, 1946).

The stress initiated by the operation was immediately continued by exposing the animals to low environmental temperature, ranging from 0° to + 4° C, for 7 hours. After this time the animals were killed, the left adrenal removed, and its cholesterol content determined.

After the operation the colonic temperature was recorded every half hour, those animals which showed marked hypothermia being for the rest of the stress period kept at room temperature.

Determination of cholesterol. — After removal of the surrounding fat, the adrenal was weighed. It was then crushed in a test-tube containing 6 ml of a 3:1 mixture of ethanol and peroxide-free ether, and boiled for 2 to 3 minutes. The extraction was repeated twice in the same way, using 5 ml of alcohol-ether each time. The extracts were combined, and total cholesterol was determined after saponification according to KELSEY (1939), but without digitonin precipitation. The modified Liebermann-Burchard reagent was used as recommended by SPERRY & BRAND (1943).

Table 18. *Cholesterol depletion of the remaining adrenal gland of unilaterally adrenalectomized-splanchnicotomized or only unilaterally adrenalectomized animals in response to cold-stress.*

Experiment	Rat No.	Sex	Body wt g	Left adrenal mg	Cholesterol mg/g adrenal
Non-stressed animals at room temperature					
Right-sided adrenalectomy and division of the left splanchnic nerve	6	♂	210	19.3	46.5
	7	♂	205	20.2	35.7
	8	♂	210	23.0	29.7
	9	♂	190	18.5	47.7
	10	♂	205	17.9	35.7
	11	♂	250	16.2	38.1
	12	♂	190	21.0	31.2
	13	♂	190	20.2	38.6
Mean ± S.E.				19.5 ± 0.7	37.9 ± 2.3
Right-sided adrenalectomy	1	♂	210	24.1	32.6
	2	♂	180	21.2	31.2
	3	♂	190	20.1	42.4
	4	♂	140	17.2	31.2
	5	♂	150	16.4	26.0
Mean ± S.E.				19.8 ± 1.4	32.7 ± 2.7
Animals exposed to cold for 7 hours					
Right-sided adrenalectomy and division of the left splanchnic nerve	14	♂	210	20.5	20.8
	16	♂	265	25.6	15.8
	17	♂	215	21.1	27.5
	18	♂	205	21.1	18.5
	20	♂	230	21.2	18.4
	22	♂	230	26.9	13.4
	24	♂	205	20.4	20.9
	26	♂	230	25.0	12.6
Mean ± S.E.				22.7 ± 0.9	18.5 ± 1.7
Right-sided adrenalectomy	15	♂	190	21.1	18.1
	19	♂	180	21.2	19.1
	21	♂	200	23.0	18.5
	23	♂	180	18.2	21.0
	25	♂	140	18.3	13.7
Mean ± S.E.				20.4 ± 0.9	18.1 ± 1.2

t-test

Subject of the test	Splanchnicotomy	Intact splanchnic nerves	Difference	Degrees of freedom	P
Non-stressed animals	37.9±2.3	32.7±2.7	5.2±3.59	11	>0.1
Animals exposed to cold	18.5±1.7	18.1±1.2	0.4±2.38	11	>0.1

Subject of the test	No stress	Exposure to cold	Difference	Degrees of freedom	P
Splanchnicotomized animals	37.9±2.3	18.5±1.7	19.4±2.84	14	<0.001
Non-splanchnicotomized animals	32.7±2.7	18.1±1.2	14.6±2.93	8	<0.005

RESULTS

CONTROL EXPERIMENTS

In order to check that division of the splanchnic nerve did not prevent the adrenal cortical response to stress, normal males were subjected either to division of the left splanchnic nerve simultaneously with removal of the right adrenal, or to right-sided adrenalectomy only. Eight or nine days after the operation half of the animals of each series were exposed to the cold for 7 hours, and then killed. The other animals were killed at the same time but without preceding exposure to cold. In the non-stressed animals the mean cholesterol concentration in the remaining left adrenal was found to be 37.9±2.3 mg/g adrenal in the unilateral adrenalectomized and splanchnicotomized animals, and 32.7±2.7 mg/g adrenal in those adrenalectomized only. After cold-stress there was a significant decrease in the cholesterol content of the adrenal gland, the mean for the animals in these two groups now being 18.5±1.7 and 18.1±1.2 mg/g adrenal, respectively (Table 18).

In another series containing 12 animals, 8 males and 4 females, the cholesterol content of the left adrenal was determined after cold-stress immediately following right-sided adrenalectomy with

Table 19. *Adrenal cholesterol depletion in response to cold-stress in normal animals. Right adrenal removed before and left adrenal after exposure to cold.*

Rat No.	Sex	Body wt g	Adrenals mg		Cholesterol mg/g adrenal		Cholesterol depletion per cent	Absolute cholesterol depletion
			Right	Left	Right	Left		
N 2	♂	215	14.8	17.0	27.0	18.8	30.4	20.0
7	♂	190	15.1	17.6	35.1	14.8	57.8	50.8
8	♂	150	12.5	15.6	42.4	19.9	53.1	41.4
9	♂	145	12.7	14.6	45.7	16.4	64.1	58.8
10	♂	150	12.9	15.6	37.2	17.9	51.9	41.8
11	♂	160	12.1	15.2	31.4	13.8	56.1	44.8
12	♂	140	12.9	13.5	37.2	24.4	34.4	31.4
13	♂	170	13.4	15.8	41.8	22.2	46.9	37.4
Mean ± S.E.					37.2 ± 2.2	18.5 ± 1.3	49.3 ± 4.1	40.8 ± 4.2
N 3	♀	155	18.0	21.6	28.9	11.6	59.9	51.8
4	♀	150	19.8	24.4	36.9	9.0	75.6	69.4
5	♀	150	18.2	20.5	26.4	12.7	51.9	45.8
6	♀	170	23.3	27.8	39.1	18.3	53.2	44.2
Mean ± S.E.					32.8 ± 3.1	12.9 ± 2.0	60.2 ± 5.4	52.8 ± 5.8

A significant difference was found between the cholesterol concentration of the right and left adrenals for males and for females, calculated according to the difference method. The following values were obtained:

Males: $\bar{d} = 18.7$ S.E. $(\bar{d}) = 2.22$
Degrees of freedom = 7 $P < 0.001$

Females: $\bar{d} = 19.9$ S.E. $(\bar{d}) = 3.03$
Degrees of freedom = 3 $P < 0.01$

simultaneous division of the left splanchnic nerve. The absolute decrease in cholesterol concentration varied between 20.0 and 58.8 per cent with a mean of 40.8 ± 4.2 per cent for the males, and between 44.2 and 69.4 per cent with a mean of 52.8 ± 5.8 per cent for the females (Table 19).

Thus, division of the splanchnic nerve, inhibiting the secretion of adrenaline from the adrenal medulla, did not prevent the release of ACTH from the anterior pituitary gland in response to stress.

The right and left adrenals, respectively, from 10 intact animals were pooled, each pool extracted separately, and analyzed for its content of cholesterol. The value (mg/g adrenal) for the left pool was found to be 3.5 per cent higher than that for the right.

ANIMALS WITH HYPOTHALAMIC LESIONS

In a series of 18 animals, 8 males and 10 females, the hypothalamic lesions were located in the region of the paraventricular nuclei, in 5 cases causing their complete destruction. The remaining animals showed a residuum of magnocellular cells varying between 240 and 2 144 cells.

In response to cold-stress the absolute decrease in cholesterol content of the left adrenal varied between 9.7 and 74.0 per cent (Table 20). For those 5 animals with complete destruction of the paraventricular nuclei the decrease in cholesterol concentration was 11.0, 16.8, 20.5, 21.2, and 33.9 per cent, respectively. In 2 other animals, having 240 and 248 magnocellular cells left in the paraventricular nuclei, the stress-induced decrease in cholesterol was 39.1. and 39.7 per cent, respectively.

The animals in this group constituted one of the earlier series in which the lesions were somewhat more extensive than in later series, as is also indicated by the accompanying protocols.

HL 224: Periventricular lesions which in the region above the optic chiasma caused marked destruction of the mediobasal tissue under the floor of the third ventricle. The medial portion of the right supraoptic nucleus was slightly destroyed, while the left supraoptic nucleus was not directly involved in the lesions. These extended caudally into the anterior end of the ventromedial nuclei, spared the median eminence, and terminated at a level corresponding to the middle portion of this latter structure. The paraventricular nuclei were completely destroyed.

HL 230: Periventricular lesions fusing above the roof of the third ventricle, and under its floor extending towards the midline, resulting in pronounced dilatation of the ventricular system. The supraoptic nuclei were not directly involved in the lesions. At the level of and immediately behind the posterior end of the optic chiasma the lesions extended down to the base of the brain, causing limited tissue damage. Caudally the lesions extended into the anterior portion of the ventromedial nuclei, in the basal direction closely approaching the dorsal part of the median eminence. Rapidly decreasing in size the lesions terminated at a level corresponding to the middle portion of the median eminence. The paraventricular nuclei were completely destroyed.

HL 241: Large, periventricular lesions fusing above and below the third ventricle, at the latter level causing marked destruction of the mediobasal tissue above and immediately behind the optic chiasma. The supraoptic nuclei were not directly involved in the lesions. In their caudal extension the lesions destroyed the anterior half of the ventromedial nuclei together with the anterior parts of the dorsomedial nuclei and dorsal hypothalamic areas, basally

Table 20. *Adrenal cholesterol depletion in response to cold-stress in animals with electrolytic lesions in the region of the paraventricular nuclei. Right adrenal removed before and left adrenal after exposure to cold.*

Rat No.	Sex	Days after op	Body wt g	Adrenals mg		Cholesterol mg/g adrenal		Cholesterol depletion per cent	Absolute cholesterol depletion	Remaining magnocellular cells in the comb. parav. nuclei
				Right	Left	Right	Left			
HL 241	♀	86	165	19.8	27.4	41.4	26.6	35.7	11.0	0
230	♂	56	175	12.9	14.9	59.7	43.0	28.0	16.8	0
223	♂	55	185	10.7	11.8	50.5	36.4	27.9	20.5	0
250	♀	96	205	25.1	29.6	28.3	18.9	33.2	21.2	0
224	♂	55	285	17.4	18.5	39.1	24.3	37.9	33.9	0
248	♀	88	190	21.5	25.3	36.3	19.4	46.6	37.1	240
239	♀	83	220	24.2	29.3	21.9	10.9	50.2	39.7	248
247	♀	88	190	19.0	22.4	22.6	16.1	28.8	16.0	368
254	♂	54	180	13.8	15.6	39.9	27.6	30.8	21.8	448
249	♂	96	240	22.7	27.6	24.7	11.2	54.7	44.9	496
262	♂	48	210	13.4	14.0	28.4	21.4	24.6	21.3	496
238	♀	83	165	24.5	30.1	29.4	21.6	26.5	9.7	668
240	♀	83	190	28.1	29.9	16.4	10.4	36.6	32.5	852
251	♀	96	230	19.8	26.0	19.2	3.8	80.2	74.0	884
258	♂	95	190	15.5	20.8	38.7	19.2	50.4	33.4	940
259	♂	95	225	15.6	20.7	42.9	24.2	43.6	25.1	1724
260	♂	96	180	11.5	15.5	32.2	18.1	43.8	24.2	1872
244	♀	86	170	28.6	33.6	28.7	12.2	57.5	50.1	2144

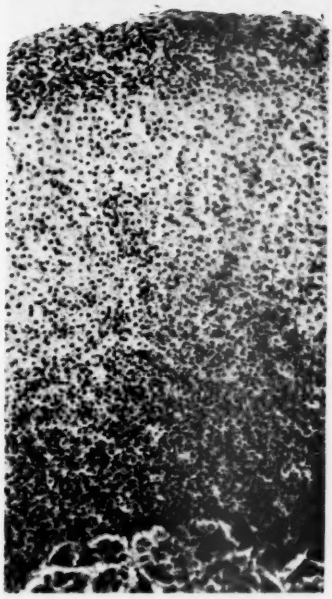


Fig. 15. Adrenal cortex of Rat HL 366, which has complete destruction of the paraventricular nuclei. Haematoxylin–eosin. × 113.

approaching the lateral border of the median eminence. They terminated at a level corresponding to the middle portion of the median eminence. The paraventricular nuclei were completely destroyed.

HL 262: Limited lesions in the region of the paraventricular nuclei, chiefly occupying a position immediately outside the lower part of the third ventricle, and causing marked dilatation of the ventricular system. The supraoptic nuclei were not directly involved in the lesions, which extended down to the base of the brain within a limited area behind the optic chiasma. Otherwise, there was only slight destruction of the mediobasal tissue under the floor of the third ventricle. Caudally the lesions extended for a short distance into the dorsal part of the anterior end of the ventromedial nuclei, terminating at a level corresponding to the middle portion of the median eminence. The partially destroyed paraventricular nuclei contained 496 magnocellular cells.

In order to study the effect of the operative intervention on the ability of the pituitary gland to respond to a cold-stress with increased release of ACTH, hypothalamic lesions were placed so

that they would not destroy any structure that is as yet considered part of the neural mechanism controlling the adrenocorticotrophic secretion from the anterior pituitary gland. In 4 animals with lesions in the preoptic region, caudally extending to the anterior end of the supraoptic nuclei, leaving intact the paraventricular nuclei and the structures behind that region, the absolute decrease in adrenal cholesterol concentration after cold-stress was found to be 31.9, 36.2, 39.3 and 46.2 per cent, respectively. Thus, the operation did not by itself prevent the acute release of ACTH from the anterior pituitary gland.

The hypophyses were in no case directly involved in the lesions, and microscopically the anterior pituitary glands appeared normal.

Adrenal glands of animals with complete destruction of the paraventricular nuclei appeared microscopically normal after routine staining with haematoxylin and eosin, after fat-staining with Sudan black, and on histochemical demonstration of cholesterol according to SCHULTZ. Fig. 15 shows a haematoxylin-eosin stained section from an adrenal gland of an animal with destruction of the paraventricular nuclei.

DISCUSSION

The results of the present experiments suggest that the paraventricular nuclei are not concerned with the secretion of ACTH from the anterior pituitary gland. This would be in accordance with the finding of MCCANN (1953), who in a series of 10 rats with electrolytic lesions in the anterior hypothalamus, in 3 cases causing complete destruction of the paraventricular nuclei, found an overall normal mean adrenal ascorbic acid depletion of the adrenal gland in response to operative stress. However, the ascorbic acid determinations in the 3 animals with destruction of the paraventricular nuclei were not treated separately. That the paraventricular nuclei are not involved in the neural control of adrenocorticotrophic secretion was also assumed by ANAND, RAGHUNATH, DUA & MOHINDRA (1954) on the basis of their finding that the paraventricular nuclei were spared by lesions effective in preventing the eosinopenic response to stress, and situated medially in the anterior hypothalamus and in the anteromedial

part of the median eminence. However, they did not check this assumption on animals with actual, localized destruction of the nuclei.

As mentioned in the Introduction to this section, the release of ACTH in response to stress is prevented by hypothalamic lesions located in the median eminence, the posterior tuber cinereum or mammillary body. Therefore, the variation observed in the present experiments in the depletion of adrenal cholesterol after exposure to cold does not appear to be due to the partial or complete degeneration of the paraventriculoneurohypophysial fibre system, but rather to a varying degree of destruction, by the fairly large lesions, of nerve fibres situated under the floor of the third ventricle and running to the primary plexus of the hypophysial portal vessels in the median eminence. Such lesions, then, would cause a partial interruption of the neural link in the postulated neurovascular mechanism controlling the secretion of ACTH from the anterior pituitary gland.

SUMMARY

Lesions completely or partially destroying the paraventricular nuclei did not block the release of ACTH from the pituitary gland in response to cold-stress. Thus, these nuclei do not seem to be concerned with the control of adrenocorticotrophic secretion from the anterior pituitary gland.

C. THE PARAVENTRICULAR NUCLEI AND THE REGULATION OF THE SECRETION OF GONADOTROPHIC HORMONES

Evidence has been produced of the existence of a hypothalamic neural control of the gonadotrophic secretion from the anterior pituitary gland. Electric stimulation of the tuber cinereum in the unanaesthetized rabbit has thus been shown to be followed by an increased release of gonadotrophic hormone from the adenohypophysis, resulting in ovulation and the formation of haemorrhagic follicles (HARRIS, 1948 a).

DEY and his co-workers (DEY, FISHER, BERRY & RANSON, 1940, DEY, 1941, 1943, BROOKHART, DEY & RANSON, 1941, DEY, LEININGER & RANSON, 1942, ALPHIN & DEY, 1944) studied the effects of hypothalamic lesions on the gonadotrophic function of the pituitary gland in guinea-pigs. They showed that large lesions situated behind the optic chiasma caused a marked decrease in the secretion of luteinizing hormone, leading to the development of large ovarian follicles, which, however, failed to rupture and to form corpora lutea. Lesions located in the region of the median eminence, on the other hand, inhibited the total gonadotrophic secretion to such an extent that gonadal atrophy ensued. HILLARP (1949 a), who made hypothalamic lesions in the rat, found the region controlling the secretion of the luteinizing hormone to be located in the anterior hypothalamic area immediately anterior and ventral to the paraventricular nucleus, and that destruction of this region resulted in a state of constant oestrus with the accompanying typical ovarian changes in the form of the development of large follicles and the absence of corpora lutea formation. A similar state was induced by means of small, symmetrical, basal lesions located caudally to the paraventricular nuclei, and probably interrupting a superficial fibre system running from the anterior hypothalamic area towards the hypophysial stalk.

The results of these types of experiment thus suggest an influence of the central nervous system on the gonadotrophic secretion from the adenohypophysis. It should then be expected that transection of the hypophysial stalk would lead to gonadal atrophy because of a greatly diminished release of the pituitary gonadotrophic hormones. In earlier investigations such intervention has, however, given highly discordant results (see HARRIS, 1955), but it now appears probable that in addition to technical imperfections this disagreement might be due to overlooking of the possible regeneration of the hypophysial portal vessels. HARRIS (1950) thus showed that when revascularization of the anterior pituitary gland with portal vessels was effectively prevented, section of the stalk was invariably followed by gonadal atrophy. Further evidence of the importance of the hypophysial portal system for the maintenance of a normal reproductive function was furnished by the experiments of HARRIS & JACOBSOHN (1952). They demonstrated that hypophysectomized female rats bearing anterior pituitary grafts under the median eminence regained normal oestrus cycles parallel with the re-establishment of a hypophysial portal blood supply, while grafts under the temporal lobe of the brain, though viable, were functionally inactive.

The hypothalamic neural control of the gonadotrophic secretion from the anterior pituitary gland thus seems to be mediated via the hypophysial portal vessels, and the present experiments were performed in order to ascertain whether the paraventriculoneurohypophysial fibre system constitutes part of the neural link of this neurovascular mechanism.

EXPERIMENTAL

A large number of female rats with electrolytic lesions in the region of the paraventricular nuclei were studied.

After the infliction of the hypothalamic lesions oestrus cycles were recorded by daily vaginal smears taken in different periods of 2 to 3 weeks' duration.

When in pro-oestrus, some of the animals were placed among males, accepted coitus being checked on the following day by

searching for sperms in the vaginal smear. The length of the gestation period could thus be determined.

The ovaries and pituitary glands were examined microscopically.

RESULTS

Oestrus cycles. – Female rats with localized hypothalamic lesions completely or partially destroying the paraventricular nuclei displayed normal oestrus rhythm. Thus, such lesions did not cause a state of constant oestrus, but since they were placed close to the region controlling the secretion of the luteinizing hormone from the adenohypophysis or the fibre system running from that region to the pituitary stalk (see HILLARP, 1949 a), they sometimes happened to involve one of these structures, too, with a resulting state of constant oestrus. Such animals did not become pregnant.

Gestation period. – Operated animals showing normal oestrus rhythm could become pregnant, and after gestation periods of normal length, 21 to 23 days, they delivered apparently normal young.

Ovaries. – The gonads were of normal weight and of normal microscopic appearance, both as regards the development of follicles and the formation of corpora lutea.

Pituitary gland. – Microscopically the anterior lobe was of normal appearance.

Thus, electrolytic lesions completely or partially destroying the paraventricular nuclei did not prevent the secretion of the follicle-stimulating or luteinizing hormone from the anterior pituitary gland.

SUMMARY

Female rats with electrolytic lesions completely or partially destroying the paraventricular nuclei did not show any alterations in ovarian function referable to a disturbance in the regulation of the secretion of the gonadotrophic hormones from the anterior pituitary gland.

Chapter VI

THE PARAVENTRICULAR NUCLEI AND THE REGULATION OF THE SECRETION OF THE POSTERIOR PITUITARY HORMONES

Convincing evidence has been produced of the essential importance of the hypothalamoneurohypophysial system for the normal water balance of the body.

In their extensive work on the neurohumoral regulation of water balance FISHER, INGRAM & RANSON (1938) clearly showed that bilateral interruption of the supraopticohypophysial tract in front of the median eminence results in diabetes insipidus because of the consequent disappearance of the antidiuretic hormone.

By using the remote control technique of stimulation HARRIS (1947) found that in the conscious rabbit, stimulation of the supraopticohypophysial tract, the median eminence, and the infundibular stem or process, resulted in a temporary inhibition of a water diuresis in the hydrated animal. Direct stimulation of the supraopticohypophysial tract was further followed by the appearance in the urine of small quantities of an antidiuretic substance (HARRIS, 1948 b).

These investigations, together with several others, references to which may be found in the monograph by G. W. HARRIS (HARRIS, 1955), show the participation of the hypothalamoneurohypophysial system in the regulation of the production and secretion of the antidiuretic hormone.

Further, it is generally accepted that also the production and secretion of the oxytocic hormone are controlled by the hypothalamoneurohypophysial system. FISHER & INGRAM (1936) thus found that interruption of the nerve fibres to the posterior pituitary gland was followed by a loss of oxytocin in this part of the

neurohypophysis. By recording the uterine response, HARRIS (1947, 1948 c) demonstrated that electric stimulation of the supraopticohypophysial tract in the rabbit caused a release of oxytocin from the posterior pituitary gland. Milk ejection, which is another criterion of oxytocic secretion (TURNER & COOPER, 1941, PETERSEN, 1942, 1944, LINZELL, 1950, WHITTLESTONE, 1950, 1952, CROSS, 1951, 1953, ANDERSSON, 1951 c, CROSS & VAN DYKE, 1953), could be elicited in the lactating sheep, goat and rabbit by electric stimulation in the region of the supraoptic nuclei (ANDERSSON, 1951 a and b, CROSS, 1955). Further, experiments on the rabbit by CROSS & HARRIS (1952) showed that stimulation of the supraopticohypophysial tract in the median eminence or of the infundibular stem caused flow of milk from cannulated teats, while interruption of the nervous connections between the hypothalamus and the posterior lobe by suitably placed hypothalamic lesions abolished the milk-ejection reflex. Milk ejection could, however, be elicited by intravenous injection of posterior pituitary extract immediately before the nursing period. Similar results concerning the necessity of the hypothalamoneurohypophysial fibre connections for the release of oxytocin, as judged by the milk-ejection response, were obtained by HARRIS & JACOBSOHN (1952) and BENSON & COWIE (1956).

There are two theories concerning the site of origin and the mode of formation of the posterior pituitary hormones.

RANSON and his group (FISHER, INGRAM & RANSON, 1938) assumed that these hormones were elaborated by the neural division of the pituitary gland, probably by the pituicytes, which they presumed to be innervated by nerve fibres belonging to the supraopticohypophysial fibre system. Thus, in the atrophied posterior lobes of cats with diabetes insipidus produced by interruption of the supraopticohypophysial tract in front of the median eminence, they found signs of degeneration of the pituicytes along with a great loss of unmyelinated nerve fibres. Assays of such glands showed an almost total absence of antidiuretic, vasopressor, and oxytocic activities.

There is, however, no obvious histologic evidence of any secretory cells within the neurohypophysis.

The other theory, based mainly on cytologic studies, is

that of neurosecretion, advanced by ERNST SCHARRER and his co-workers (SCHARRER, 1930, 1937, SCHARRER & GAUPP, 1933, SCHARRER & SCHARRER, 1937, 1940). According to this hypothesis, the posterior pituitary hormones are formed in the ganglion cells of the magnocellular hypothalamic nuclei and transported along the axis cylinders to the posterior pituitary gland, where they are stored until released into the circulation (SCHARRER & SCHARRER, 1945, BARGMANN, 1949 a and b, 1951, BARGMANN & HILD, 1949, BARGMANN & SCHARRER, 1951, SCHARRER & SCHARRER, 1954 a and b).

Much work in this field is based on the discovery of BARGMANN (1949 a) that the neurosecretory material within the hypothalamoneurohypophysial fibre system can be stained with GOMORI's chrome alum haematoxylin method. The Gomori-stainable material has been widely regarded as a glycolipoprotein bearer-substance for the posterior pituitary hormones (SCHIEBLER, 1951, 1952 a and b, HILD & ZETLER, 1953 a). Recently, however, other histochemical evidence has been brought forward, suggesting the neurosecretory material to be a protein rich in cystine, and probably representing the hormones themselves (SLOPER, 1954, 1955, ADAMS & SLOPER, 1955, 1956).

ABEL (1924), working on the sheep, was the first to discover an antidiuretic substance in the hypothalamus. This finding was later confirmed both in extracts from the whole hypothalamus and from the region of the supraoptic nuclei (SATO, 1928, TRENDELENBURG, 1928, MELVILLE & HARE, 1945, HILD & ZETLER, 1951 a and b, 1952 a and b, KOVÁCS & BACHRACH, 1951, VOGT, 1953). HILD & ZETLER found posterior pituitary hormones in all parts of the hypothalamoneurohypophysial system as well as a close parallelism between the amount of hormone and the amount of neurosecretory material.

After transection of the hypophysial stalk an accumulation of Gomori material has been observed proximal to the site of operation, while distally the neurohypophysis is depleted both of neurosecretory material and hormonal content (HILD, 1951 a and b, SCHARRER & WITTENSTEIN, 1952, HILD & ZETLER, 1953 b).

Further, it has been shown that the increased release of antidiuretic hormone produced by dehydration is reflected in the neurohypophysis as a decrease in the amount of neurosecretory

material, which will again be found in ordinary amount on restitution of normal hydration (ORTMANN, 1951, HILD & ZETLER, 1953 b, LEVEQUE & SCHARRER, 1953).

Thus, there is good evidence for the assumption that the magnocellular cells in the supraoptic and paraventricular nuclei are concerned with the elaboration of the posterior pituitary hormones.

There is a problem still to be settled, however, *viz.* whether the antidiuretic and the oxytocic principles are two separate hormones, or whether they only represent two different activities of one and the same hormone. This question is, of course, of the utmost importance for the understanding of the function of the supraoptic and the paraventricular neurons, respectively.

Investigations dealing with this problem have been reviewed by WARING & LANDGREBE (1950) and by HARRIS (1955). HARRIS states that, though many experimental findings indicate the existence of two separate hormones, it is at present not possible to form a definite opinion on this point.

The most important argument for the one-hormone hypothesis has been that the antidiuretic and oxytocic principles in different animals always run parallel, so that conditions causing a decrease of one of the principles in the posterior pituitary gland will also cause a decrease of the other. Recent experiments, using electric stimulation of the supraopticohypophysial tract (HARRIS, 1947, 1948 b and c), and indirect reflex stimulation of the hypothalamus (CROSS, 1951, ABRAHAMS & PICKFORD, 1954) have, however, provided indirect evidence supporting the view that the two hormones might be secreted in other proportions than those in which they are present in the posterior lobe. DICKER & TYLER (1953 a and b) further found that during lactation the oxytocic activity in the posterior pituitary gland decreased independently of the vasopressor activity. Further support for the two-hormones hypothesis is the finding of VOGT (1953), who showed that in the dog the ratio of the antidiuretic to the oxytocic activity was not the same in the hypothalamus as in the posterior lobe.

The experiments with fractionation of posterior pituitary extracts and with isolation of two active principles seem in better agreement with the two-hormones hypothesis, but it is difficult to preclude

the possibility of the active fractions being broken off from a single large parent molecule during the separation procedures.

If two separate hormones are formed and secreted by the hypothalamoneurohypophysial system, it seems possible that the two magnocellular hypothalamic nuclei might have separate functions. It appears to be a widely accepted view, however, that these two nuclei have a common function. But this problem has received very little attention in earlier investigations, the two magnocellular nuclei and the neurohypophysis being taken together under the common heading of the supraopticohypophysial system and treated as one entity. Lack of closer analysis of a possible differentiation in function between the nuclei seems mainly to be bound up with the difficulty in making localized stimulations or lesions in this region.

The two main fibre tracts within the hypothalamoneurohypophysial system, running from the anterior hypothalamus, are the supraopticoneurohypophysial and the paraventriculoneurohypophysial tracts. Analysis of the effect of a loss of only one of these fibre systems requires destruction of its nuclei of origin. In the median eminence the two tracts have joined to form part of the hypothalamoneurohypophysial tract. Therefore, lesions within and immediately in front of this region cannot selectively interrupt nerve fibres belonging to only one of these fibre systems. The term "interruption of the supraopticohypophysial tract" after lesions placed basally in and in front of the median eminence is thus not adequate. Investigations hitherto performed with the use of lesion technique therefore provide no possibility of judging whether the operative effect depends on interruption of nerve fibres belonging to only one or to both of the magnocellular fibre systems.

Investigations for any occurrence of different functions of the two magnocellular systems have apparently never been performed with the use of lesion technique. On the other hand, experiments have been carried out with electric stimulation of the region of the two magnocellular nuclei.

Harris (1947) thus showed that electric stimulation of the supraopticohypophysial tract in the rabbit gave a marked antidiuretic response, while stimulation in the vicinity of the

paraventricular nucleus was followed neither by antidiuresis, nor by any increased uterine activity. In the lactating sheep and goat ANDERSSON (1951 b) observed that electric stimulation of the region of the supraoptic nuclei resulted in milk ejection, and later ANDERSSON & MCCANN (1955 a and c) reported stimulation in or adjacent to the paraventricular or supraoptic nuclei to be followed by both antidiuresis and milk ejection. A milk-ejection response to stimulation in the region of the paraventricular or supraoptic nuclei was also obtained by CROSS (1955), but stimulation of the paraventricular nucleus elicited the response only after adrenalectomy.

These results, then, do not agree as far as the effect of stimulation of the paraventricular nucleus is concerned. It seems, however, as if the release of oxytocin could be elicited by the paraventricular nucleus as well as by the supraoptic nucleus. But this does not admit of the conclusion that both nuclei have the same function since nothing is known about the magnitude of the amount secreted. Further, it cannot be excluded that stimulation in the region of the supraoptic nucleus might also imply stimulation of some fibres from the paraventricular nucleus. The possibility that nerve fibres from the supraoptic nucleus run to the paraventricular nucleus, as discussed in chapter IV, must also be borne in mind. Such a fibre connection might explain the antidiuretic response obtained *also* on stimulation of the paraventricular nucleus.

It is thus apparent that the possibility of separate functions of the two magnocellular hypothalamic nuclei must be taken into account, but that experiments hitherto on record do not admit of any valid conclusions in this respect. It was therefore considered of interest to investigate the effect of lesions localized in the paraventricular nuclei on the production and secretion of the posterior pituitary hormones.

A. EFFECTS OF HYPOTHALAMIC LESIONS IN THE REGION OF THE PARAVENTRICULAR OR SUPRAOPTIC NUCLEI ON WATER METABOLISM

Factors influencing the activity of the hypothalamoneurohypophysial system and thereby also the secretion of the antidiuretic hormone have been studied more closely by VERNEY and his colleagues. Under quiescent conditions the secretion of antidiuretic hormone, according to VERNEY's theory, is regulated by the osmotic pressure of the blood.

Ingestion of water by mouth thus produces a decrease in the osmotic pressure of the blood and thereby a depression of neurohypophysial activity, resulting in increased diuresis, starting when the amount of antidiuretic hormone present in the blood before the administration of water has been excreted or inactivated. Therefore, water diuresis may be regarded as a condition of physiologic diabetes insipidus (KLISIECKI, PICKFORD, ROTHSCHILD & VERNEY, 1933 a and b).

Compatible with the theory that hydration causes an inhibition of the secretion of antidiuretic hormone were the results of those experiments which showed that dehydration is followed by signs of release of this hormone from the posterior pituitary gland. Thus, GILMAN & GOODMAN (1937) found that the urine from rats, dehydrated either by water deprivation or by the administration of hypertonic saline, contained an antidiuretic substance, presumably of pituitary origin. This substance was not found in the urine from normal animals or from dehydrated hypophysectomized animals, and like the antidiuretic hormone it was destroyed on hydrolysis.

The activation of the neurohypophysis by deprivation of water or by administration of hypertonic saline with consequent release of

antidiuretic hormone, was then confirmed in the rat (Boylston & Ivy, 1938), cat (Martin, Herrlich & Fazekas, 1939, Ingram, Ladd & Benbow, 1939) and dog (Hare, Hickey & Hare, 1941, Hare, Hare & Phillips, 1943, Chambers, Melville, Hare & Hare, 1945).

Administration of hypotonic solutions thus decreases the secretion of antidiuretic hormone from the posterior pituitary gland, while administration of hypertonic solutions increases it.

By detailed analysis of the effect of intracarotid injection of hypertonic solutions, Verney (1946, 1947, 1948) showed that activation of the neurohypophysis is dependent on an increase of the osmotic pressure of the blood. He assumed the existence of autonomic receptive elements, called osmoreceptors, functionally linked with the neurohypophysis, situated in the area supplied by the internal carotid artery, and reacting to osmotic changes in the blood. The exact position of these osmoreceptors is not known. Hare (1947) assumed that they are situated in the hypothalamus. A normal antidiuretic response was thus obtained after isolation of the diencephalon by transection of the midbrain, extirpation of the cervical sympathetic trunks, and section of the first three cranial nerves, while the response was prevented by section of the hypophysial stalk. By intracarotid infusion of hypertonic solutions before and after intradural ligation of one internal carotid, Jewell & Verney (1953) could show that the site of the osmoreceptors is to be sought in the procencephalon. As a hypothesis Verney (1947) suggested that the small vesicles observed by him within the field of the supraoptic nucleus might constitute the postulated osmoreceptors. Jewell (1953) made a more detailed study of these structures and arrived at similar conclusions. Since the supraoptic nucleus is pervaded by a very rich capillary network (Finley, 1940), extremely good conditions are provided for intimate contact between blood and ganglion cells.

Whether also the paraventricular nuclei partake in the regulation of water balance is not known with certainty. In six cats with complete or almost complete destruction of the paraventricular nuclei Fisher, Ingram & Ranson (1938) could not demonstrate any increase in fluid exchange. However, they did not state in how many cases a complete destruction of the nuclei was achieved,

nor did they perform any determination of the number of remaining magnocellular cells in the paraventricular nuclei in those cases where their destruction was not complete.

STEVENSON (1949), on the other hand, working on the rat, found that electrolytic lesions in or near the ventromedial nuclei, located behind the paraventricular nuclei at the level of the median eminence, produced marked disturbance in water balance. The site of the lesions, however, had only been estimated by mere inspection of the surface of the hypothalamus, and no histologic study was made of their extension. Animals with such lesions drank less in proportion to their food intake than did intact controls, and showed increased tubular reabsorption of water together with a marked delay in the excretion of a water load. STEVENSON assumed the upset in water balance to be the result of a chronic state of relative dehydration, possibly due to destruction of nervous elements regulating water intake. The dehydration would thus produce an increased secretion of antidiuretic hormone resulting in increased tubular reabsorption of water. In further support of this hypothesis STEVENSON, WELT & ORLOFF (1950) demonstrated a significant increase in the concentration of serum sodium in such animals.

Neurohypophysectomy in the dog, producing well defined diabetes insipidus without any obvious signs of anterior pituitary deficiency, is followed by pronounced depression of renal function with an approximately 50 per cent reduction of the glomerular filtration rate, renal plasma flow and tubular excretory maximum for para-aminohippurate (HANDLEY & KELLER, 1950, DEMUNBRUN, KELLER, LEVKOFF & PURSER, 1954). Further DEMUNBRUN *et al.* showed that administration of posterior pituitary total extract (Pituitrin), the oxytocic fraction of such extract (Pitocin), or DU VIGNEAUD's purified oxytocin, restored the depressed renal functions to normal levels, while administration of the pressor fraction (Pitressin) was without effect. They therefore attributed the depression of renal functions after extirpation of the neurohypophysis to a deficiency of oxytocin. Of special interest in this connection is the finding reported in Section C of this chapter, *viz.* that loss of the paraventriculoneurohypophysial fibre system

sometimes appears to be followed by a reduction in the amount of oxytocin in the posterior pituitary gland.

The purpose of the present experiments was to determine whether destruction of the paraventricular nuclei can cause a disturbance in water balance severe enough to be revealed by measurement of the daily urine volume or by abnormal disposing of an excess load of water or salt solutions.

METHODS

Urine collection. – The daily urine volume of each operated rat was measured for a period not less than one week. The animals were placed in individual metabolism cages, 25 cm in diameter, and provided with a network bottom. Glass funnels were placed under the cages, and the urine was collected in glass vessels. In order to prevent contamination of the urine with faeces, pear-shaped glass balls were placed under the openings of the funnels. The collected urine volume was measured each morning at the same time.

The diet consisted of corn and water *ad libitum*. The water was kept in round glass flasks equipped with a relatively narrow tube through which the animals could drink without any water being spilt.

Fluid loads. – The diuresis was recorded after:

a: The administration of a single dose of tap water, 0.5 per cent and 3 per cent sodium chloride solution in a dose of 5 per cent of body weight.

b: The administration of two doses of distilled water or 2 per cent sodium chloride solution, given at hourly intervals, and each dose consisting of 3 ml per 100 sq cm of body surface, which latter was calculated according to the formula of BENEDICT (1934, 1938):
Surface area in sq cm $= K \cdot w^{2/3}$, where $K = 9.1$, and w is the weight in grams.

For 16 hours prior to the load the animals were deprived of food but allowed to drink *ad libitum*.

The fluid was warmed to body temperature, and was then administered by stomach tube.

Since the animals with hypothalamic lesions were often irritable, they were given a small dose of ether immediately before the administration of the fluid load, just sufficient to produce a slight dizziness that made them easy to handle. According to BIRNIE, EVERSOLE, BOSS, OSBORN & GAUNT (1950), the amount of antidiuretic substance demonstrable in the serum of rats is essentially the same in etherized as in non-anaesthetized animals.

The controls consisted of intact males of about the same age and the same weight as the operated animals. As a rule 4 animals with hypothalamic lesions and 2 controls were used for each loading experiment.

The time taken for administration of the fluid to 6 rats was usually about 12 to 14 minutes.

Immediately after the administration of the fluid load the animals were placed in small, individual metabolism cages, 12 cm in diameter. Here, too, contamination of the urine with faeces was prevented by pear-shaped glass balls under the funnels. The urine volume excreted was measured every fifteenth minute for 3 or 5 hours. During the experiment the animals were deprived of food and water.

The course of the diuresis was recorded by determination, at 30-minute intervals, of the percentage of the load excreted. This made it possible to form an opinion on the ability of the operated animals to dispose of an excess fluid load as compared with the normal animals.

Determination of sodium and potassium. – The serum sodium concentration was determined in animals that had been deprived of food but not of water for 16 hours before they were killed. Under ether anaesthesia the left carotid artery was exposed, the vessel cut through, and the blood allowed to run freely into a centrifuge tube containing no anti-coagulant. During the collection of the blood the central end of the cut vessel was facing the inner wall of the centrifuge tube, pressure against the neck being avoided in order to prevent contamination of the collected blood with sodium from the surrounding tissues. After coagulation the

blood was centrifuged, the serum separated and analyzed for sodium.

Determinations were also made of the amount of sodium and potassium in the urine collected for 3 hours after the administration of 2 per cent sodium chloride solution by stomach tube.

The sodium and potassium concentrations were determined with a lithium internal standard Perkin Elmer flame photometer model number 52 A.

RESULTS

I. DETERMINATION OF THE DAILY URINE VOLUME

Determinations were made of the daily urine volume of animals with electrolytic lesions in the region of the paraventricular or in the region of the supraoptic nuclei. Both the operated animals and the controls were males.

Three series of operated animals were used, each series having its own controls. Since the possibility of seasonal variation in diuresis and in the disposing of excess fluid loads cannot be excluded, the various series in this and in the following sections were not taken together, but treated separately in the analysis of the results. This was considered necessary even though it makes it more difficult to survey the material.

Series 1. – This series consisted of 44 animals with lesions in the region of the paraventricular nuclei, in 26 causing complete, and, in the remaining 18, partial destruction of these nuclei. The control material consisted of 14 animals.

Measurement of the daily urine volume of 5 of the animals was started about 3 weeks after the operation; of the remainder, after more than 1 month.

For the animals with complete destruction of the paraventricular nuclei the daily urine volume was found to be 2.9 ± 0.12 ml, and for those with partial destruction of the nuclei 2.4 ± 0.11 ml. The mean value found for the controls was 3.1 ± 0.14 ml (Table 21).

Series 2. – This series consisted of 15 animals with lesions in the region of the paraventricular nuclei. In 8 animals there was complete, and in 7 animals partial destruction of the nuclei. The control material consisted of 18 animals.

Table 21. *Daily urine volume of animals with complete or partial destruction of the paraventricular nuclei.*

Type	Number of animals	Period of observation in days	Daily urine volume ml
Series 1			
Controls	14	7	3.1 ± 0.14
Complete destruction of the parav. nuclei	26	7	2.9 ± 0.12
Partial destruction of the parav. nuclei	18	7	2.4 ± 0.11
Series 2			
Controls	18	7	3.5 ± 0.20
Complete destruction of the parav. nuclei	8	7	3.0 ± 0.20
Partial destruction of the parav. nuclei	7	7	2.4 ± 0.16

t-test

Subject of the test	Difference	Degrees of freedom	P
Series 1			
Normal animals — animals with complete destruction of the parav. n.	0.2 ± 0.19	>120	>0.1
Normal animals — animals with partial destruction of the parav. n.	0.7 ± 0.17	>120	<0.001
Animals with complete — animals with partial destruction of the parav. n.	0.5 ± 0.17	>120	<0.005
Series 2			
Normal animals — animals with complete destruction of the parav. n.	0.5 ± 0.30	>120	<0.1
Normal animals — animals with partial destruction of the parav. n.	1.1 ± 0.31	>120	<0.001
Animals with complete — animals with partial destruction of the parav. n.	0.6 ± 0.27	>120	<0.025

The interval between the operation and the beginning of the urine measurements was more than 4 months.

The mean found for the daily urine volume for the animals with complete destruction of the paraventricular nuclei was 3.0 ± 0.20 ml, and for the animals with partial destruction of the nuclei 2.4 ± 0.16 ml. For the controls the mean urine volume was 3.5 ± 0.20 ml (Table 21).

Thus, lesions completely or partially destroying the paraventricular nuclei did not produce diabetes insipidus or any tendency towards such a condition.

Series 3. — This series consisted of 15 animals with lesions in the region of the supraoptic nuclei, and with a varying degree of destruction of these nuclei. In all the animals the paraventricular nuclei were left intact.

During two different periods the animals were placed in metabolism cages for the recording of the daily urine output. The first period, which lasted for 26 days, was started 12 and 13 days after the operation, while the second, during which the animals were kept in the cages for 14 days, was started 90 and 91 days after the operation. During this latter recording period 2 of the animals, HL 464 and 469, were killed for determination of the amount of vasopressin in the posterior pituitary gland.

In the cat the latent period preceding the onset of the permanent phase of diabetes insipidus lies between 10 and 13 days (Fisher, Ingram & Ranson, 1938). Clark (cited from R. Gaupp, 1941) gave the length of this period in the rat as 2 to 6 days after the infliction of the hypothalamic lesions.

The subjective judgement of the degrees of destruction of the supraoptic nuclei is given in Table 22, and it should be stressed that no great degree of precision is claimed for these estimations. The degree of destruction of the supraoptic nuclei showed the following distribution:

Marked	destruction	of	the	supraoptic	nuclei:	6	cases
Moderate	»	»	»	»	» :	5	cases
Slight	»	»	»	»	» :	4	cases

By marked destruction is meant a pronounced bilateral reduction in the number of cells in the supraoptic nuclei. The terms

Table 22. *Daily urine volume of animals with partial destruction of the supraoptic nuclei.*

Series 3			
Rat No.	Degree of destruction of the supraoptic nuclei	Daily urine volume, ml	
		First period of observation	Second period of observation
HL 461	Marked	6.6 ± 0.52	8.5 ± 0.73
462	»	3.2 ± 0.70	3.1 ± 0.24
464	»	17.0 ± 3.09	8.3 ± 1.15
465	»	12.6 ± 1.43	5.2 ± 0.95
469	»	22.4 ± 2.49	9.1 ± 1.53
478	»	8.2 ± 0.63	4.3 ± 0.52
460	Moderate	3.6 ± 0.27	3.8 ± 0.75
466	»	1.1 ± 0.23	1.4 ± 0.17
468	»	1.9 ± 0.35	6.2 ± 0.89
476	»	2.5 ± 0.34	2.5 ± 0.41
477	»	4.9 ± 0.62	7.3 ± 1.09
471	Slight	3.3 ± 0.34	1.8 ± 0.24
472	»	5.4 ± 0.76	5.1 ± 0.68
474	»	3.6 ± 0.32	3.2 ± 0.27
475	»	2.6 ± 0.26	2.7 ± 0.17

"moderate" and "slight" destruction represented gradually lower degrees of destruction of the nuclei.

In 3 animals HL 464, 465 and 469, for which the largest urine volumes were recorded, the supraoptic nuclei were markedly destroyed, leaving a residuum of 4 856, 2 596 and 3 616 magnocellular cells, respectively. In the intact animals the mean number of cells in the supraoptic nuclei was found to be 13 668 ± 483 cells (see chapter IV).

Fully developed diabetes insipidus was not seen in any of the animals in this series. During the first recording period, however, the animals HL 464, 465 and 469 showed a distinct increase in urine output with maximum values of 58, 32 and 46 ml/day, respectively. During the first 5 days the animal HL 469 showed normal urine output, but afterwards polyuria developed with relatively high values sometimes alternating with lower values. During the remaining 21 days of the recording period the urine output noted for this animal was more than 20 ml/day for 5 days and more than 30 ml/day for 10 days (Table 22). On the other

Table 23. *Water load response of animals with electrolytic lesions com*

Type	Number of animals	Number of cells in the parav. nuclei	Per cent excreted			
			30 min	60 min	90 min	120 min
Series A						
Controls	16		0	8.3±2.39	33.8±3.48	56.9±4.2
Complete destruction of the parav. nuclei	14	0	0.6±0.46	4.4±1.70	21.9±2.60	36.1±3.8
Series B						
Controls	40		0.2±0.09	11.6±1.17	39.1±1.52	66.1±1.6
Complete destruction of the parav. nuclei	14	0	1.3±0.54	9.2±1.81	32.8±3.84	54.0±4.1
Marked destruction of the parav. nuclei	7	339±70	0.7±0.70	13.9±4.51	39.7±5.92	63.5±6.6
Moderate destruction of the parav. nuclei	9	1112±123	0.3±0.26	12.6±3.01	37.6±3.98	57.0±2.6
Series C						
Controls	16		0.1±0.07	9.8±1.54	35.6±2.80	56.9±3.9
Complete destruction of the parav. nuclei	7	0	0	5.8±1.67	23.2±2.61	37.9±3.9
Moderate destruction of the parav. nuclei	7	946±89	0.2±0.20	9.0±2.01	22.2±3.79	36.2±5.2

Rate of excretion of a water load by animals with electrolytic lesions in the region of the paraventricular nuclei.

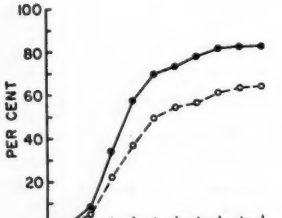


Fig. 16. Series A.

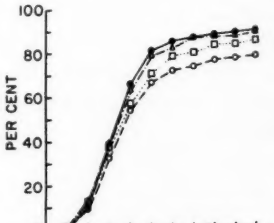
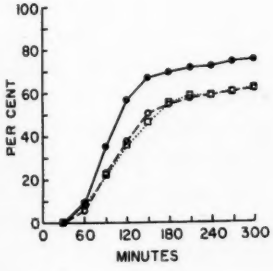


Fig. 17. Series B.

electrolytic lesions completely or partially destroying the paraventricular nuclei.

	Series A						
	Per cent excreted						
90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min
.8±3.48	56.9±4.24	69.0±3.48	72.4±3.12	77.3±2.69	80.4±2.70	81.0±2.63	81.2±2.54
.9±2.60	36.1±3.88	48.6±4.85	53.3±5.08	55.2±5.37	59.8±4.92	61.8±4.36	62.1±4.36
	Series B						
1±1.52	66.1±1.66	81.3±1.43	85.5±1.35	87.2±1.59	88.7±1.62	89.3±1.64	90.2±1.64
8±3.84	54.0±4.11	66.5±3.89	72.0±4.35	73.9±4.47	76.6±4.52	77.5±4.43	78.3±4.25
7±5.92	63.5±6.64	78.8±7.81	82.1±6.80	87.6±7.75	87.6±7.75	87.6±7.75	89.1±8.05
6±3.98	57.0±2.65	70.5±2.39	78.2±2.28	80.0±2.01	83.7±2.90	83.7±2.90	85.3±2.56
	Series C						
6±2.80	56.9±3.96	67.0±2.19	69.7±1.78	71.7±1.93	72.1±1.96	74.0±1.63	75.1±1.63
2±2.61	37.9±3.90	50.3±4.79	54.7±5.75	57.4±5.22	58.8±4.49	60.8±4.15	61.8±4.82
2±3.79	36.2±5.23	46.3±5.27	55.5±5.95	58.5±6.47	59.1±6.27	60.2±6.19	62.1±5.45



Controls.

Complete destruction of the paraventricular nuclei.

Marked destruction of the paraventricular nuclei.

Moderate destruction of the paraventricular nuclei.

Fig. 18. Series C.

Table 24. *Response to loading with 0.5 per cent sodium chlor[illegible]olution of [illegible] partially destroying [illegible]araventricu*

Type	Number of animals	Number of cells in the parav. nuclei	Per cent excreted			
			30 min	60 min	90 min	120 min
Series A						
Controls	20		0	13.8±1.71	28.6±1.72	34.6±1.8
Complete destruction of the parav. nuclei	11	0	0	2.6±0.61	18.6±2.24	22.8±2.6
Series B						
Controls	35		0.3±0.25	13.1±1.93	38.7±1.91	49.3±1.7
Complete destruction of the parav. nuclei	13	0	0	12.7±2.10	32.5±2.48	39.8±2.3
Marked destruction of the parav. nuclei	7	339±70	0.1±0.13	12.2±3.50	37.6±4.89	40.4±3.1
Moderate destruction of the parav. nuclei	9	1112±123	1.0±1.01	15.4±3.14	36.7±3.72	42.2±3.0

Rate of excretion of a load of 0.5 per cent sodium chloride solution by animals with electrolytic lesions in the region of the paraventricular nuclei.

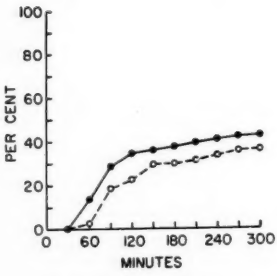


Fig. 19. Series A.

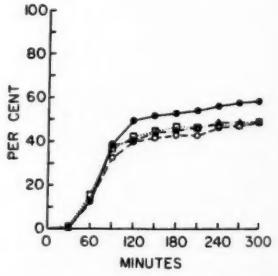


Fig. 20. Series B

•—•—•—• Controls.
○ - - ○ - - ○ Complete destruction of the paraventricular nuclei.
△-·-△-·-△ Marked destruction of the paraventricular nuclei.
□ · · · □ · · · □ Moderate destruction of the paraventricular nuclei.

um chlor[illegible]olution of animals with electrolytic lesions completely or [illegible]stroying [illegible]paraventricular nuclei.

90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min
			Series A				
			Per cent excreted				
3.6±1.72	34.6±1.80	36.0±1.78	37.7±2.37	39.4±2.46	40.9±2.24	42.2±2.44	42.6±2.46
3.6±2.2[illegible]	22.8±2.67	29.4±2.36	29.9±2.30	31.2±2.25	33.5±1.86	35.7±2.13	36.1±2.11
			Series B				
5.7±1.91	49.3±1.75	51.7±1.63	52.9±1.70	54.0±1.74	56.1±1.73	57.6±1.87	58.3±1.76
2.5±2.49	39.8±2.36	41.3±2.52	42.7±2.76	42.7±2.76	46.2±2.91	46.9±3.10	48.9±2.91
4.6±4.80	40.4±3.15	43.5±3.15	44.9±3.22	46.3±3.59	48.5±4.02	48.5±4.02	48.5±4.02
5.7±3.72	42.2±3.08	44.8±2.58	46.5±2.63	46.5±2.63	47.3±2.47	47.7±2.58	48.9±2.40

hand, during the second measuring period, performed 3 months after the operation, these 3 animals showed a return to normal or almost normal values (Table 22).

Animals with marked but not complete destruction of the supraoptic nuclei may thus show a varying, though not extreme, degree of polyuria, lasting for several weeks.

II. RESPONSE TO EXCESS FLUID LOADS

The experimental material consisted of 6 series of animals, A–F. Normal males were used as controls.

Those animals in which the lesions were placed in the region of the paraventricular nuclei received the loads at different times between 2 and 10 months after the operation; those with the lesions located in the region of the supraoptic nuclei, between 2 and 5 months after the operation.

The animals received tap water or distilled water, 0.5 per cent saline, and 3 or 2 per cent saline, by stomach tube. When the animals received more than one type of fluid load, they were given water, hypotonic and hypertonic solution in the order

mentioned. The interval between the administration of the different types of fluid loads varied between one week and three weeks.

In the first 3 series the animals were given a single dose corresponding to 5 per cent of body weight. In the remaining series they received two doses at hourly intervals, each dose consisting of 3 ml per 100 sq cm of body surface.

In the first 3 series the course of the diuresis was followed for 5 hours. In the remaining series it was followed for 3 hours after the administration of the second dose.

ANIMALS WITH LESIONS IN THE REGION OF THE PARAVENTRICULAR NUCLEI

1. *Fluid load corresponding to 5 per cent of body weight and given as a single dose*

Three series, A–C, were used for these experiments. All of the operated animals were males. The results are summarized in Tables 23, 24 and 25.

As is apparent from Table 23 and Figs. 16, 17 and 18 all 3 series showed a delay in the excretion of an excess water load by those animals that showed complete destruction of the paraventricular nuclei. The animals with marked or moderate destruction of the nuclei did not differ appreciably from the normal animals as regards the rate of unloading in Series B, but in Series C also those animals with only moderate destruction of the paraventricular nuclei showed a delay in the excretion of the water load of the same degree as the animals with complete destruction of these nuclei.

With a load of 0.5 per cent sodium chloride solution (Series A and B) the animals with complete destruction of the paraventricular nuclei showed a somewhat delayed excretion of the fluid as compared with the normal animals (Table 24, Figs. 19 and 20). The course of excretion of the hypotonic solution was roughly the same for the animals with partial destruction of the nuclei as for those with complete destruction.

With a load of 3 per cent sodium chloride solution (Series A and B) no appreciable difference was found between normal

animals and animals with complete or partial destruction of the paraventricular nuclei (Table 25, Figs. 21 and 22). For the animals with complete and moderate destruction, respectively, the values were as a rule somewhat lower than for the controls and for the animals with marked destruction of the paraventricular nuclei.

2. *Fluid loads corresponding to 3 ml per 100 sq cm of body surface and administered twice at hourly intervals*

Two series, D and E, were used for these experiments. Series D consisted of both male and female rats, Series E of females only. The results are summarized in Tables 26 and 27.

Among the male rats in Series D a water load was excreted at about the same rate both in animals with complete and in animals with partial destruction of the paraventricular nuclei, and somewhat more slowly than in the normal males (Table 26, Fig. 23 a). In Series D no appreciable difference was found in this respect between the operated females with intact, partially or completely destroyed paraventricular nuclei (Fig. 23 b). On the other hand, the 3 female rats with complete destruction of the paraventricular nuclei in Series E showed a distinct delay in the excretion of the water load as compared with the intact animals or the animals with partial destruction of these nuclei (Table 26, Fig. 24).

No difference was found in the rate of excretion of loads of 2 per cent sodium chloride solution (Series D, Table 27, Fig. 25 a and b) between the normal males and the males with complete or partial destruction of the paraventricular nuclei. The female rats with lesions located in the region of the paraventricular nuclei showed a somewhat higher rate of excretion than the operated female controls with electrolytic lesions situated anteriorly and laterally to the paraventricular nuclei.

ANIMALS WITH LESIONS IN THE REGION OF THE SUPRAOPTIC NUCLEI

All of the operated animals in this series (Series F) were males. The lesions were located in the region of the supraoptic nuclei and caused their partial destruction; in no case, however, extensive

Table 25. *Response to loading with 3 per cent sodium chlor... solution ... partially destroying ... paraventr...*

Type	Number of animals	Number of cells in the parav. nuclei	Per cent excreted			120 min
			30 min	60 min	90 min	
Series A						
Controls	17		12.2±5.15	15.7±4.95	31.5±3.67	51.3±...
Complete destruction of the parav. nuclei	9	0	13.8±8.45	18.4±8.45	30.3±9.40	43.8±...
Series B						
Controls	29		14.8±3.63	21.0±3.71	40.6±3.74	61.1±...
Complete destruction of the parav. nuclei	12	0	41.8±7.62	45.0±7.93	53.2±6.27	63.0±...
Marked destruction of the parav. nuclei	6	367±76	39.9±11.58	43.9±10.58	53.9±9.02	69.2±...
Moderate destruction of the parav. nuclei	9	1112±123	42.4±7.39	45.3±7.05	56.2±5.25	70.2±...

Rate of excretion of a load of 3 per cent sodium chloride solution by animals with electrolytic lesions in the region of the paraventricular nuclei.

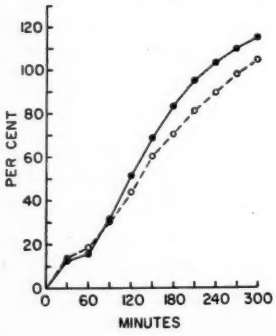


Fig. 21. Series A.

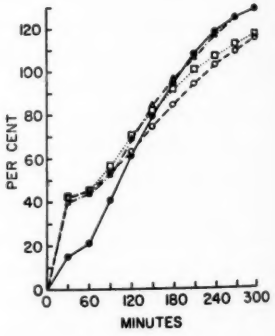


Fig. 22. Series B.

•—•—•—• Controls.

○ - - ○ - - ○ Complete destruction of the paraventricular nuclei.

△-·-△-·-△ Marked destruction of the paraventricular nuclei.

□···□···□ Moderate destruction of the paraventricular nuclei.

solution of animals with electrolytic lesions completely or paraventricular nuclei.

Series A						
Per cent excreted						
120 min	150 min	180 min	210 min	240 min	270 min	300 min
51.3±3.18	68.8±3.44	83.5±4.05	95.4±4.73	103.5±5.40	110.1±5.50	115.4±5.70
43.8±10.08	60.2±10.74	70.3±10.72	81.1±11.55	89.9±11.68	98.3±11.17	105.0±11.03
Series B						
61.1±3.79	79.3±3.73	94.8±3.71	108.3±3.67	118.8±3.80	125.5±3.86	129.9±3.86
63.0±5.20	74.4±4.68	84.6±4.22	94.3±3.79	103.2±3.90	109.7±4.47	116.0±4.78
69.2±7.70	84.3±7.42	96.6±6.28	106.7±6.85	117.2±7.07	125.4±6.85	129.7±6.58
70.2±4.55	82.0±4.56	92.0±5.17	101.1±5.08	107.3±5.40	113.0±6.00	117.6±6.22

enough to produce such a degree of degeneration of the supra-opticoneurohypophysial fibre connections as to cause diabetes insipidus. The paraventricular nuclei were not directly involved in the lesions.

The animals received two doses of fluid, each consisting of 3 ml per 100 sq cm of body surface, and given at hourly intervals. They first received distilled water, and 13 to 20 days later 8 of the animals were also given loads of 2 per cent sodium chloride solution. In this second loading experiment the same controls were used as for Series D, which was also being studied at that time.

The 3 animals HL 464, 465 and 469, which showed temporary polyuria as a consequence of the operation, were not included in this series.

When loaded with distilled water or with 2 per cent sodium chloride solution, the animals with partial destruction of the supraoptic nuclei showed the same rate of excretion as the controls (Tables 28 and 29, Figs. 26 and 27).

Table 26. *Response to loading with distilled water of animals with electrolytic lesions completely or partially destroying the paraventricular nuclei.*

Type	Number of animals	Sex	Number of cells in the parav. nuclei	Per cent excreted					
				30 min	60 min	90 min	120 min	150 min	180 min
Series D									
Intact controls	19	♂		7.2 ± 1.08	20.5 ± 1.88	33.8 ± 2.42	45.1 ± 2.26	53.6 ± 1.90	56.2 ± 1.55
Operated controls, lesions rostral or lateral to the parav. nuclei	4	♀	2436 ± 197	5.9 ± 2.28	18.9 ± 3.33	32.6 ± 4.75	46.1 ± 4.17	52.8 ± 4.27	60.0 ± 2.42
Complete destruction of the parav. nuclei	3	♂	0	4.8 ± 2.77	16.9 ± 4.10	27.5 ± 6.85	37.1 ± 7.68	42.6 ± 9.43	45.4 ± 9.62
Complete destruction of the parav. nuclei	3	♀	0	4.3 ± 2.16	17.2 ± 2.16	29.2 ± 2.31	38.5 ± 3.31	48.3 ± 4.64	57.8 ± 1.53
Partial destruction of the parav. nuclei	5	♂	920 ± 168	3.8 ± 1.87	12.7 ± 2.06	23.7 ± 3.90	35.1 ± 4.50	40.7 ± 4.95	48.0 ± 5.98
Partial destruction of the parav. nuclei	4	♀	834 ± 104	2.8 ± 1.71	15.9 ± 2.55	30.2 ± 3.83	39.3 ± 3.42	47.9 ± 2.66	54.4 ± 3.82
Series E									
Intact controls	16	♂		6.3 ± 1.34	21.7 ± 2.06	38.2 ± 2.45	51.2 ± 2.70	59.4 ± 2.17	62.5 ± 1.87
Complete destruction of the parav. nuclei	3	♀	0	2.4 ± 1.96	8.6 ± 3.46	19.9 ± 3.11	27.6 ± 2.24	35.3 ± 3.26	39.9 ± 2.83
Partial destruction of the parav. nuclei	10	♀	1320 ± 164	8.2 ± 1.89	23.7 ± 2.96	38.4 ± 3.22	48.3 ± 3.30	53.9 ± 3.24	56.6 ± 2.70

Rate of excretion of a load of distilled water by animals with electrolytic lesions in the region of the paraventricular nuclei.

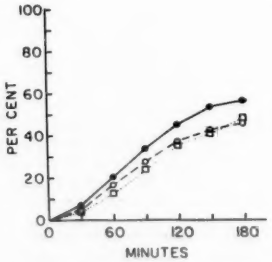


Fig. 23 a. Males of Series D.

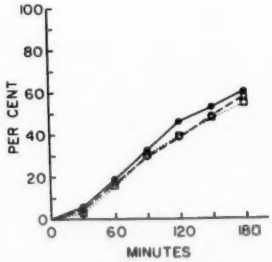


Fig. 23 b. Females of Series D.

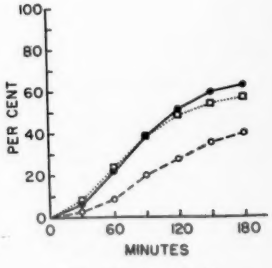


Fig. 24. Series E.

•—•—•—• Controls.

○ - - ○ - - ○ Complete destruction of the paraventricular nuclei.

□ · · · □ · · · □ Partial destruction of the paraventricular nuclei.

Table 27. *Response to loading with 2 per cent sodium chloride solution of animals with electrolytic lesions completely or partially destroying the paraventricular nuclei.*

Series D									
Type	Number of animals	Sex	Number of cells in the parav. nuclei	Per cent excreted					
				30 min	60 min	90 min	120 min	150 min	180 min
Intact controls	20	♂		5.0 ± 0.48	23.0 ± 1.76	39.2 ± 2.54	50.0 ± 2.50	57.6 ± 2.68	62.9 ± 2.61
Operated controls, lesions rostral or lateral to the parav. nuclei	4	♀	2436 ± 197	19.5 ± 11.60	26.0 ± 11.00	35.3 ± 9.90	41.2 ± 9.53	47.7 ± 8.91	52.4 ± 8.74
Complete destruction of the parav. nuclei	4	♂	0	21.8 ± 6.75	31.0 ± 7.43	43.1 ± 11.31	50.0 ± 12.00	57.8 ± 11.83	61.4 ± 13.29
Complete destruction of the parav. nuclei	3	♀	0	17.4 ± 8.47	32.0 ± 3.54	37.8 ± 3.11	46.3 ± 4.48	55.8 ± 4.12	61.9 ± 5.33
Partial destruction of the parav. nuclei	5	♂	920 ± 168	22.5 ± 7.65	33.7 ± 8.08	44.3 ± 10.53	51.3 ± 10.89	59.9 ± 9.94	65.0 ± 9.67
Partial destruction of the parav. nuclei	5	♀	987 ± 296	21.9 ± 5.49	33.1 ± 8.28	44.7 ± 8.28	52.8 ± 8.58	58.0 ± 7.80	61.7 ± 8.41

Rate of excretion of a load of 2 per cent sodium chloride solution by animals with electrolytic lesions in the region of the paraventricular nuclei.

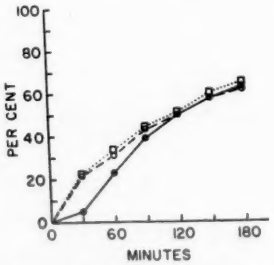


Fig. 25 a. Males of Series D.

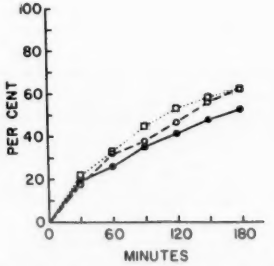


Fig. 25 b. Females of Series D.

•—•—•—• Controls.

○ - - ○ - - ○ Complete destruction of the paraventricular nuclei.

□ · · · □ · · · □ Partial destruction of the paraventricular nuclei.

Table 28. *Response to loading with distilled water of animals with electrolytic lesions in the region of the supraoptic nuclei.*

Series F							
Type	Number of animals	Per cent excreted					
		30 min	60 min	90 min	120 min	150 min	180 min
Controls	25	6.7 ± 0.89	20.8 ± 1.53	34.9 ± 1.97	46.6 ± 1.85	55.5 ± 1.66	58.0 ± 1.38
Marked destruction of the supraoptic nuclei	4	3.6 ± 2.10	16.3 ± 2.06	30.9 ± 1.55	44.5 ± 3.00	54.8 ± 3.37	59.4 ± 2.72
Moderate destruction of the supraoptic nuclei	8	4.3 ± 1.82	17.3 ± 3.14	32.5 ± 4.04	46.1 ± 4.37	55.7 ± 4.33	60.5 ± 3.68
Slight destruction of the supraoptic nuclei	8	5.3 ± 2.18	19.1 ± 2.49	34.5 ± 3.02	47.7 ± 2.66	54.1 ± 2.88	59.0 ± 1.35

Table 29. *Response to loading with 2 per cent sodium chloride solution of animals with electrolytic lesions in the region of the supraoptic nuclei.*

Series F							
Type	Number of animals	Per cent excreted					
		30 min	60 min	90 min	120 min	150 min	180 min
Controls	20	5.0 ± 0.48	23.0 ± 1.76	39.2 ± 2.54	50.0 ± 2.50	57.6 ± 2.68	62.9 ± 2.61
Partial destruction of the supraoptic nuclei	8	8.8 ± 1.16	22.9 ± 1.08	38.9 ± 1.09	47.2 ± 1.20	55.0 ± 1.94	61.6 ± 1.84

Rate of excretion of a load of distilled water by animals with electrolytic lesions in the region of the supraoptic nuclei.

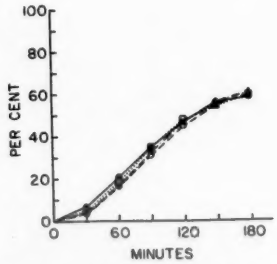


Fig. 26. Series F (Table 28).

•—•—•—• Controls.

○ - - ○ - - ○ Marked destruction of the supraoptic nuclei.

△-·-△-·-△ Moderate destruction of the supraoptic nuclei.

□ · · · □ · · · □ Slight destruction of the supraoptic nuclei.

Rate of excretion of a load of 2 per cent sodium chloride solution by animals with electrolytic lesions in the region of the supraoptic nuclei.

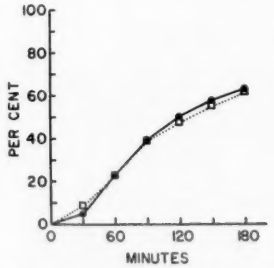


Fig. 27. Series F (Table 29).

•—•—•—• Controls.

□ · · · □ · · · □ Partial destruction of the supraoptic nuclei.

III. DETERMINATION OF SERUM SODIUM

About 6 months after operation the serum sodium concentration was determined in 8 male rats with complete destruction of the paraventricular nuclei. Fourteen normal males served as controls. No difference was found between the experimental and the control animals, the mean concentration of serum sodium in the two groups being 149.6 ± 2.10 and 149.6 ± 1.07 mEq/l, respectively.

While rats with hypothalamic lesions in or near the ventromedial nuclei have an increased concentration of serum sodium, possibly because of a chronic state of relative dehydration (Stevenson, Welt & Orloff, 1950), the results of the present investigation showed that animals with complete, localized destruction of the paraventricular nuclei have a normal concentration of sodium in the serum.

IV. THE URINARY SODIUM AND POTASSIUM EXCRETION AFTER LOADING WITH HYPERTONIC SODIUM CHLORIDE SOLUTION

In connection with the administration of 2 per cent sodium chloride solution to all the animals in Series D and 8 of the animals in Series F, the concentration of sodium and potassium was determined in the urine volume collected during the first 3 hours after loading. The amount of sodium excreted was calculated both as the percentage of the dose given, and as mg sodium per 100 sq cm of body surface. For potassium, only this latter, relative value was determined.

Also now, the intact male rats served as controls for the operated males, and the females with hypothalamic lesions not involving the paraventricular nuclei served as controls for the female experimental rats.

The results are summarized in Table 30.

No significant difference was found in the amount of sodium excreted between the animals with lesions located in the region of the paraventricular or supraoptic nuclei and their respective controls. While there was likewise no significant difference in the excreted amount of potassium between the animals with complete destruction of the paraventricular nuclei or partial destruction of the supraoptic nuclei and the control rats, the

Table 30. *Urinary sodium and potassium, after loading with 2 per cent sodium chloride solution, of animals with electrolytic lesions in the region of the paraventricular or supraoptic nuclei.*

Series D and F					
Type	Number of animals	Sex	Percentage Na excreted of dose given	mg Na/3 hours/ 100 sq cm of body surface	mg K/3 hours/ 100 sq cm of body surface
Intact controls	20	♂	23.8 ± 0.78	28.5 ± 0.93	5.2 ± 0.25
Operated controls, lesions rostral or lateral to the parav. nuclei	4	♀	20.5 ± 2.80	24.6 ± 3.40	5.8 ± 0.94
Complete destruction of the parav. nuclei	4	♂♀	20.5 ± 5.00	24.6 ± 6.00	5.7 ± 0.95
Complete destruction of the parav. nuclei	3	♂♀	20.2 ± 1.21	24.3 ± 1.41	6.4 ± 0.95
Partial destruction of the parav. nuclei	5	♂♀	21.5 ± 3.58	25.8 ± 4.26	7.9 ± 0.78
Partial destruction of the parav. nuclei	5	♀	22.0 ± 2.05	26.3 ± 2.46	7.2 ± 0.68
Partial destruction of the supraoptic nuclei	8	♂	22.4 ± 0.58	26.8 ± 0.70	5.7 ± 0.25

animals with only partial destruction of the paraventricular nuclei showed a tendency to excrete an increased amount of potassium in the urine. In the present small material this amount differed significantly from the controls for the males ($P < 0.001$), but not for the females ($P > 0.1$).

In these latter animals there were greater possibilities of the lesions involving neurons located laterally to the paraventricular nuclei than in the animals with complete destruction of the nuclei, where the lesions were situated close to the ventricular wall. This might possibly explain the differences in the amount of potassium excreted by these two groups.

DISCUSSION

In view of the necessity of making a clear distinction between the supraopticoneurohypophysial and the paraventriculoneurohypophysial systems in order to be able to demonstrate any difference in their function, it would surely be of interest to study the effect on fluid exchange of an isolated loss of the supraopticoneurohypophysial system, produced by localized, direct destruction of the supraoptic nuclei. That such experiments have not hitherto been performed is probably due to the technical difficulties involved in destroying these elongated nuclei.

This difficulty was not fully mastered in the present investigation either: thus the lesions did not cause complete destruction of the supraoptic nuclei, although a pronounced loss of cells in the nuclei was achieved. During an observation period of more than 3 weeks, starting about 2 weeks after the operation, 3 of the animals with such lesions showed a distinct tendency to excrete larger urine volumes than did normal animals or animals with lesions in the region of the paraventricular nuclei, though they did not show the picture of fully developed diabetes insipidus. It therefore appears possible that not only interruption of the hypothalamoneurohypophysial tract in front of the median eminence, but also bilateral, complete destruction of the supraoptic nuclei, leaving the paraventricular nuclei intact, will result in diabetes insipidus. The normalization of the urinary output observed later in these animals with partial destruction of the supraoptic nuclei

might possibly, in view of the neurosecretory hypothesis, be due to the remaining neurons requiring a certain time before being capable of producing the amount of antidiuretic hormone necessary in the individual case for the re-establishment of a normal tubular reabsorption of water.

The role of the paraventricular nuclei for the secretion of the antidiuretic hormone is not clear. Thus, in the rabbit electric stimulation of these nuclei does not cause any antidiuretic response (Harris, 1947), while Andersson & McCann (1955 c) reported this to be the case in the goat. In the present experiments it was shown, in accordance with the findings of Fisher, Ingram & Ranson (1938), that complete destruction of the paraventricular nuclei does not cause any increase in the fluid exchange. This finding, *viz.* that destruction of the paraventricular nuclei does not produce a state of diabetes insipidus, together with the tendency to polyuria after marked destruction of the supraoptic nuclei, without direct involvement of the paraventricular nuclei, indicate that the latter nuclei are not necessary for the normal production and secretion of the antidiuretic hormone in the rat. It might, therefore, be possible that this function is exerted only by the supraopticoneurohypophysial system in the strict sense of the term.

In the water load experiments animals with complete destruction of the paraventricular nuclei showed a delay in unloading, as compared with intact controls. The various series were examined at different times, and the results might suggest that this inability to dispose normally of a water load might be due to the absence of the paraventriculoneurohypophysial system. Since, however, animals with varying degrees of partial destruction of the paraventricular nuclei showed either normal excretion or the same delayed excretion as animals with complete destruction of these nuclei, the cause of the delay in unloading cannot be ascribed to the reduction in the number of functioning magnocellular cells within the paraventricular nuclei. The inability of the animals to rid themselves of an excess water load might instead be due to involvement in the lesions of other neurons common to these animals, probably located immediately outside the paraventricular nuclei.

Animals with varying degrees of destruction of the supraoptic nuclei disposed of a load of water as well as of hypertonic sodium chloride solution in about the same way as did normal animals. Therefore, together with those animals with lesions in the region of the paraventricular nuclei that showed normal treatment of a water load, they provided evidence that a delay in the excretion of such a load cannot be due to a non-specific operative effect.

Lesions in or near the ventromedial nuclei cause a state of chronic dehydration with accompanying increase in the concentration of serum sodium, possibly due to destruction of neural elements belonging to a "drinking centre". Animals with such lesions also show a significant reduction in the water/food intake ratio (Stevenson, 1949, Stevenson, Welt & Orloff, 1950).

Closer analysis of the extension of such a "drinking area" has shown that microinjection of hypertonic sodium chloride solution into a region located in the middle portion of the hypothalamus, somewhat behind and laterally to the paraventricular nuclei, causes drinking in the goat, as does electric stimulation of this region. In addition to a drinking response, stimulation in front of the columna fornicis descendens also produces inhibition of a water diuresis as well as milk ejection in the lactating animal; while stimulation immediately lateral to the paraventricular nuclei produces only the latter two responses (Andersson, 1952 ,1953, Andersson & McCann, 1955 a, b and c). Electrolytic destruction in the dog of a region containing this "drinking area" was followed by a hypodipsia lasting up to 14 days after the operation (Andersson & McCann, 1956). In good agreement with these findings Greer (1955), working on the rat, reported a case of violent drinking activity in response to bilateral, electric stimulation of a region situated immediately behind the paraventricular nuclei, at the lateral edge of the dorsomedial nuclei, just above the ventromedial nuclei.

In the present investigation the animals with partial destruction of the paraventricular nuclei showed a significant decrease in the daily urine output, as compared with the intact controls. The daily water intake was not measured, but the diminished excretion was probably a manifestation of a diminished water intake. In

the light of the findings of ANDERSSON and ANDERSSON & MCCANN it seems possible that these lesions destroyed part of the "drinking area" located outside the paraventricular nuclei, and that this led to a state of hypodipsia. This might also explain why the decrease in urine output was found in the animals with partial destruction of the paraventricular nuclei, and not in the animals with complete destruction of the nuclei; in the former there was a greater chance that the lesions involved nerve cells belonging to the region located laterally to the paraventricular nuclei and containing the postulated "drinking area", than in the latter where the lesions were situated close to the ventricular wall.

It was thus possible that because of the lesions the animals were in a state of slight dehydration, which would in turn have produced an increased secretion of antidiuretic hormone from the neurohypophysis. Since, however, the concentration of serum sodium as determined 3 months after the water load experiment was found to be normal in animals with complete destruction of the paraventricular nuclei, and in which a delayed water diuresis had been demonstrated 2 to 3 months after the operation, it does not seem probable that such a degree of dehydration had actually been present that this alone would have been able to explain the delay in unloading.

Neurohypophysectomy seems to be followed by depression of renal function, which can be restored by the administration of oxytocin (DEMUNBRUN, KELLER, LEVKOFF & PURSER, 1954). Since lesions in the region of the paraventricular nuclei sometimes appeared to result in a decreased amount of oxytocin in the posterior pituitary gland (see Section C of chapter VI), the delayed excretion of a water load in animals with such lesions might possibly be due to impaired renal function because of a deficiency of oxytocin.

In Section A of chapter V it was shown that complete destruction of the paraventricular nuclei does not prevent the secretion of thyrotrophic hormone from the adenohypophysis. Since administration of thyroxine to intact rats increases urine flow during water diuresis, while hypothyroid animals show a normal water load response (GAUNT, CORDSEN & LILING, 1944), any delay in the excretion of a water dose after destruction of the paraventricular nuclei cannot be due to abnormal thyroid function.

It is well known that the adrenal cortex influences water balance. Adrenalectomized rats thus show a marked delay in the excretion of a water load (GAUNT, 1944, GAUNT, BIRNIE & EVERSOLE, 1949) because of increased tubular reabsorption of water (LOTSPEICH, 1949). This upset in water metabolism might, at least in part, be explained as the result of an addition of the increased amount of antidiuretic substance in the blood, and the increased sensitivity of the organism to posterior pituitary hormones, occurring after adrenalectomy (BIRNIE, EVERSOLE, BOSS, OSBORN & GAUNT, 1950, MIRSKY, PAULISCH & STEIN, 1954). As was shown in Section B of chapter V, animals with complete destruction of the paraventricular nuclei were still, though to a somewhat less extent than normal animals, able to respond to a cold-stress with increased secretion of ACTH, leading to the release of adrenal cortical hormones, demonstrated as depletion of cholesterol in the adrenals. Therefore, a dysfunction of these glands large enough to explain the delayed excretion of a water load seems hardly likely to have been present.

Bilateral, complete destruction of the paraventricular nuclei, then, does not seem to cause any severe disturbance in water balance. Though the material was too small to admit of any valid conclusions on the metabolism of sodium and potassium, animals with such lesions did show a normal concentration of serum sodium, and a normal excretion of sodium and potassium in the urine after loads of hypertonic sodium chloride solution.

SUMMARY

In animals with lesions located in the region of the paraventricular nuclei, the daily urine volume as well as the response to loads of water and hypotonic and hypertonic sodium chloride solutions, were recorded. Apart from the administration of hypotonic solution similar experiments were performed on animals with a varying degree of direct destruction of the supraoptic nuclei.

While the daily urine volume in animals with complete destruction of the paraventricular nuclei did not significantly differ from

that in normal animals, it did in those animals with only partial destruction of the nuclei.

The animals with complete, and some of those with partial destruction of the paraventricular nuclei, excreted a water load at a slower rate than did normal animals. The decrease in urine flow was not directly related to the magnitude of the loss of magnocellular cells within the paraventricular nuclei. The common cause of the delayed excretion in these animals might instead be due to involvement in the lesions of other neurons probably located immediately outside the paraventricular nuclei. This region has been claimed to contain a postulated "drinking centre" (ANDERSSON, 1952, 1953, ANDERSSON & MCCANN, 1955 a, b and c, 1956), the destruction of which would cause hypodipsia, which in turn would result in dehydration with subsequent increase in the secretion of antidiuretic hormone from the neurohypophysis. Since the concentration of serum sodium was normal in animals with complete destruction of the paraventricular nuclei, this militates against at least any appreciable degree of dehydration. Another conceivable explanation of the inability of these animals normally to dispose of a water load might be the possible occurrence of an impairment in the renal function depending on a deficiency of oxytocin.

Loads of hypotonic sodium chloride solution were excreted by animals with complete, and by animals with partial destruction of the paraventricular nuclei at about the same rate, and in both groups somewhat more slowly than in normal animals.

The operated animals and the controls disposed of loads of hypertonic sodium chloride solution in roughly the same way.

The delay in the excretion of a water load in animals with destruction of the paraventricular nuclei does not seem to be due to dysfunction either of the thyroid or of the adrenal glands.

Animals with localized, marked, but not complete, destruction of the supraoptic nuclei showed a temporary polyuria. The possibility that the supraopticoneurohypophysial system, taken in the strict sense of the term, is alone responsible for the antidiuretic function of the neurohypophysis, is discussed.

Bilateral, complete destruction of the paraventricular nuclei, then, does not seem to cause any marked disturbance in water

balance. As to the metabolism of sodium and potassium, the experiments admit of no valid conclusions. However, animals with such lesions showed a normal concentration of serum sodium, and a normal excretion of sodium and potassium in the urine after loading with hypertonic sodium chloride solution. On the other hand, after similar loads, animals with partial destruction of the paraventricular nuclei showed a tendency to excrete a larger amount of potassium than normal animals, and this might be interpreted as due to involvement in the lesions of neurons located laterally to the paraventricular nuclei. These neurons escaped injury in the animals with complete destruction of the nuclei because of the lesions here being situated closer to the ventricular wall.

B. DETERMINATION OF THE AMOUNT OF ANTIDIURETIC-VASOPRESSOR HORMONE IN THE POSTERIOR PITUITARY GLAND IN ANIMALS WITH LESIONS IN THE REGION OF THE PARAVENTRICULAR NUCLEI

Interruption of the nerve fibres to the neurohypophysis leads to a loss of the posterior pituitary hormones in the gland. This effect can be utilized in the investigation of the role played by the paraventricular nuclei in the formation and secretion of these hormones. If the two magnocellular hypothalamic systems have different functions in this respect, an isolated destruction of one of these systems might produce a quantitative as well as a qualitative change in the hormone content of the posterior pituitary gland. This possibility was therefore investigated.

This Section deals with a study of the vasopressor activity of the posterior pituitary gland in rats with electrolytic lesions in the region of the paraventricular nuclei, causing only a slight involvement of the supraopticoneurohypophysial system. The results of similar experiments concerning the oxytocic activity of the gland are given in the following Section (page 144). A preliminary report on these studies has been published (Olivecrona, 1954).

It is widely believed that the antidiuretic and vasopressor activities are two effects of one and the same substance (cf. Waring & Landgrebe, 1950, Pickford, 1952). This substance has recently been isolated and chemically analyzed by Du Vigneaud and co-workers (Turner, Pierce & Du Vigneaud, 1951, Du Vigneaud, Lawler & Popenoe, 1953), who found the antidiuretic-vasopressor hormone to be a polypeptide containing eight amino-acids. Since an assay of the vasopressor activity of the posterior pituitary gland is simpler than an assay of the antidiuretic activity, the former test method was decided upon.

METHODS

PREPARATION OF EXTRACTS

The isolated posterior pituitary gland was prepared in the following way:

1. Immediately after the animal had been killed the hypophysis was removed, the posterior pituitary lobe isolated and dropped into cold acetone and kept in a refrigerator for 1–2 hours.
2. It was then transferred to cold redistilled peroxide-free ether and kept in the refrigerator for 1 hour.
3. The ether was poured off, and the gland was dried over *silica gel* in an evacuated desiccator.
4. The dried gland was then crushed with a glass rod in a test tube containing 0.25 per cent acetic acid.

   Extraction was usually done in 8 ml acetic acid.
5. The tube was plugged with cotton-wool and placed in a boiling water bath for 2 minutes.
6. After cooling the tube under running tap water the contents were filtered through a small dry filter paper into another glass tube.
7. The extract was then placed in a refrigerator until it was used for the assay of its vasopressor activity. This was done on the same day or the day after the animal had been killed.

The posterior lobes from operated animals were compared either with normal posterior lobes or with a standard powder, "Hypadrin" (ASTRA), prepared from ox posterior lobe material, which had been standardized by the manufacturers against the international standard, and been found to contain about 1 000 international units per gramme.

To prepare an extract from this substandard, 50 mg of the powder was transferred to a glass tube containing 5 ml of 0.25 per cent acetic acid. Extraction was done in the same way as for the isolated rat posterior lobe. The filtrate was then diluted with 0.25 per cent acetic acid so that 1 ml contained 0.1 unit.

The amount of vasopressin in normal posterior lobes was determined by assaying them against this substandard.

DETERMINATION OF THE VASOPRESSOR ACTIVITY

The dibenamine-treated rat preparation according to the method described by DEKANSKI (1952) was used. The assays were performed on males weighing between 200 and 435 grammes.

Preparation of the rat. – The animal was anaesthetized with urethane (175 mg per 100 g body weight) injected subcutaneously. After 2 hours the rat was placed in the supine position on the operation table and was fixed by its hind legs and by a length of string placed behind the upper incisors. The front legs were not secured. A midline incision was made over the throat and after retraction of the submaxillary glands and cleavage of the pretracheal muscle, the trachea was cannulated with a short polythene tube about 3 mm in outer diameter. The left external jugular vein was used for injections. It was detached from the subcutaneous tissue and ligated at its cranial end. The vein was manually engorged, an incision made with scissors, and a polythene tube with an outer diameter of 1 mm and filled with heparin inserted into it. The other end of the polythene cannula, which contained 0.05 ml, was connected with a tuberculin syringe filled with normal saline.

Heparin (2 mg per 100 g body weight) was injected through the venous cannula and washed in with saline.

Then the right carotid artery was freed from surrounding tissue and a ligature placed at its cranial end. A glass cannula with an outer diameter of 1 mm was inserted into the artery and connected to a mercury manometer with an inner diameter of 2 mm by a column of normal saline. The same arterial cannula was used in all the assays.

The writing point was made of a strip of aluminium and was attached to the upper end of a straw, the lower end of which was fitted into a float made of a small polythene rod. The float rested on the surface of the mercury and was freely movable within the glass tubing, which it fitted as closely as possible.

Each dose given was washed in with 0.08 ml of normal saline. The total volume injected was always the same for both the test and standard solutions.

A constant, basal blood pressure was obtained by intravenous

Table 31. *The amount of vasopressin in the posterior pituitary glands of normal animals.*

Rat No.	Sex	Body wt g	Amount of vasopressin units
N 35	♂	160	0.4
38	♂	305	0.8
40	♂	205	0.8
41	♂	220	0.8
42	♂	215	1.2
43	♂	200	0.8
45	♂	200	0.6
46	♂	220	0.4
47	♂	225	0.5
55	♂	295	1.1
56	♂	275	1.1
59	♂	165	0.5
151	♂	285	0.8
152	♂	250	0.8
153	♂	250	0.8
147	♀	220	0.8
148	♀	220	0.8
149	♀	210	0.8
150	♀	210	0.8
Mean ± S.E.			0.8 ± 0.05

t-test					
Subject of the test	Males	Females	Difference	Degrees of freedom	P
Amount of vasopressin	0.76 ± 0.064	0.80	−0.04 ± 0.127	17	>0.1

injections of dibenamine, which was dissolved according to the description given in DEKANSKI's paper. Four to six doses of dibenamine (0.1 mg per 100 g body weight per dose) were injected at 5 minute intervals. After the blood pressure had reached its basal level, injection of 0.3 ml of normal saline caused only a slight response due to a volume effect.

Performance of the test. — Test and standard solutions, both at room temperature, were injected through the venous cannula with a tuberculin syringe. The doses, 2 of the standard and 2 of

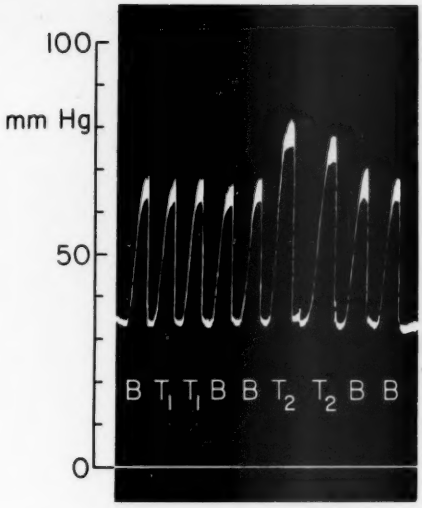


Fig. 28. Blood pressure of rat (300 g). Urethane. Dibenamine 1.50 mg. The pressor assay of a normal posterior pituitary gland (N 152) against the Hypadrin–standard.

B = Hypadrin–standard (0.0025 unit).
T_1 = 1/320 of the posterior pituitary gland.
T_2 = 1/160 of the posterior pituitary gland.

the test solution, were given at intervals of 3 to 10 minutes in series of 4 in the order A B B A. At least 2 series of 4 injections, giving the same blood pressure rise for both test and standard solutions, were recorded. Further, doses of the unknown extract, less and more potent than the standard, were also tested.

RESULTS

NORMAL ANIMALS

In a series of 19 animals, 15 males and 4 females, with a body weight ranging from 160 to 305 g (mean 230 ± 9.2 g), the amount of vasopressin found in the posterior pituitary gland varied between 0.4 and 1.2 unit, with a mean of 0.8 ± 0.05 unit (Table 31).

An illustration of a typical assay of a normal posterior pituitary gland is given in Fig. 28.

Table 32. *The amount of vasopressin in the posterior pituitary glands of animals with complete destruction of the paraventricular nuclei.*

Rat No.	Sex	Days after op	Body wt g	Amount of vasopressin
HL 311	♂	279	300	0.8 unit
313	♂	321	260	0.6 unit
332	♂	347	210	0.4 unit
349	♂	573	270	0.8 unit
397	♂	339	270	0.8 unit
336	♂	352	310	the same activity as in a normal gland
337	♂	373	345	the same activity as in a normal gland
366	♂	328	210	half the activity of a normal gland
407	♀	180	240	0.8 unit
415	♀	302	270	0.4 unit
405	♀	129	225	the same activity as in a normal gland

ANIMALS WITH HYPOTHALAMIC LESIONS

1. *Animals with complete destruction of the paraventricular nuclei*

This series consisted of 11 animals, 8 males and 3 females. They were killed 129–573 days after the operation, and their body weights varied between 210 and 345 g (mean 265 ± 12.8 g). The lesions were of the usual type, located periventricularly without direct involvement of the supraoptic nuclei.

The results of the vasopressor assays are given in Table 32.

In 7 cases the posterior pituitary glands were assayed against the "Hypadrin"-standard, and here the vasopressor activity was found to vary between 0.4 and 0.8 unit. In 4 cases they were tested against normal glands. In 3 of these the vasopressor activity was found to be the same as, and in 1 half of, that exerted by the normal lobes, which would also mean a content of about 0.4–0.8 unit.

Part of the record of the vasopressor assay of HL 415 is given in Fig. 29.

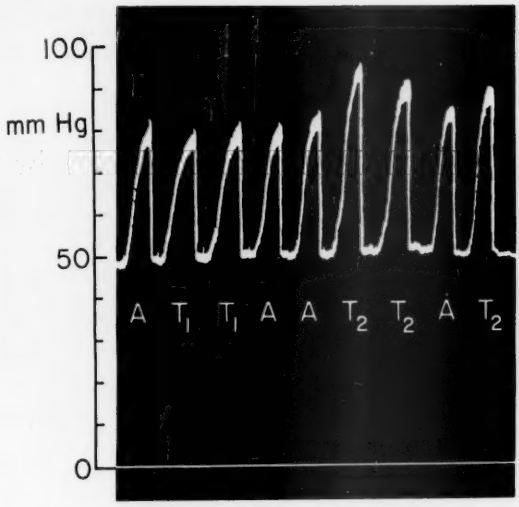


Fig. 29. Blood pressure of rat (295 g). Urethane. Dibenamine 1.77 mg. The pressor assay of the posterior pituitary gland of Rat HL 415, in which the lesions caused complete destruction of the paraventricular nuclei.

A = Hypadrin–standard (0.005 unit).
T_1 = 1/80 of the posterior pituitary gland.
T_2 = 1/50 of the posterior pituitary gland.

2. *Animals with partial destruction of the paraventricular nuclei*

In 17 animals, 9 males and 8 females, the number of remaining magnocellular cells in the combined right and left paraventricular nuclei varied between 112 and 1 384 cells. In 8 cases less than 25 per cent of the normal number of cells was left in the nuclei. The body weight of the animals, which were killed 88–571 days after the operation, ranged from 150 to 340 g (mean 255 ± 12.0 g).

The vasopressor activity of the posterior pituitary gland was assayed against the "Hypadrin"-standard in 8 cases and in 9 cases against normal glands. In the former group the glands from operated animals contained between 0.4 and 0.8 unit, and in the latter 6 showed the same and 3 half the activity of the

Table 33. *The amount of vasopressin in the posterior pituitary glands of animals with partial destruction of the paraventricular nuclei.*

Rat No.	Sex	Days after op	Body wt g	Number of cells in the parav. nuclei	Amount of vasopressin
HL 404	♀	127	220	112	the same activity as in a normal gland
376	♀	345	240	268	0.5 unit
347	♂♀	569	265	344	0.8 unit
403	♀	114	215	488	the same activity as in a normal gland
377	♀	334	215	528	the same activity as in a normal gland
321	♂	366	290	564	the same activity as in a normal gland
356	♀	571	190	616	0.4 unit
326	♂	313	275	620	the same activity as in a normal gland
384	♂	185	300	788	the same activity as in a normal gland
335	♂	366	255	812	half the activity of a normal gland
431	♀	268	290	864	0.4 unit
315	♂♀	328	310	1024	0.6 unit
373	♀	331	215	1024	half the activity of a normal gland
396	♂	366	300	1032	0.8 unit
309	♂♂♀	277	340	1328	0.8 unit
392	♂♀	358	270	1332	0.8 unit
430	♀	88	150	1384	half the activity of a normal gland

normal posterior lobes (Table 33). As can further be seen in this table, the amount of vasopressin in the posterior pituitary gland was not dependent on the number of functioning magnocellular cells within the paraventricular nuclei.

Animals with localized lesions, completely or partially destroying the paraventricular nuclei, thus showed a normal or slightly decreased amount of vasopressin in the posterior lobe.

3. *Animals with lesions located laterally to or behind the paraventricular nuclei*

In a series of 7 rats, 1 male and 6 females, with body weights ranging from 130 to 275 g (mean 220 ± 17.1 g), the lesions, in 3

Table 34. *The amount of vasopressin in the posterior pituitary glands of animals with lesions located laterally to or behind the paraventricular nuclei.*

Rat No.	Sex	Days after op	Body wt g	Location of the lesions	Number of cells in the parav. nuclei	Amount of vasopressin units
HL 353	♀	561	130	Lateral to the parav. nuclei	1600	0.4
357	♀	573	230	»	1712	0.8
441	♀	284	215	»	2052	0.8
440	♀	274	215	Behind the parav. nuclei	2180	0.8
439	♀	267	225	»	2600	0.8
446	♂	288	275	»	2796	0.4
437	♀	249	250	»	2912	0.8
Mean ± S.E.					2265 ± 196	0.7 ± 0.07

Table 35. *The amount of vasopressin and oxytocin in the posterior pituitary glands of two animals with lesions in the region of the supraoptic nuclei.*

Rat No.	Sex	Days after op	Body wt g	Number of cells in the parav. nuclei	Number of cells in the supraoptic nuclei	Amount of vasopressin units	Amount of oxytocin units
HL 464	♂	99	290	1888	4856	0.8	0.8
469	♂	99	265	2608	3616	0.6	0.4

of the animals, were situated laterally to and in 4 behind the paraventricular nuclei. The animals were killed 249–573 days after the operation. The number of magnocellular cells in the combined right and left paraventricular nuclei varied between 1 600 and 2 912, with a mean of 2 265 ± 196 cells.

The posterior pituitary glands of all the animals were assayed against the "Hypadrin"-standard, and their amount of vasopressor activity was found to be 0.4–0.8 unit (mean 0.7 ± 0.07 unit) (Table 34).

Animals with such lesions thus showed a normal content of vasopressin in the posterior pituitary gland.

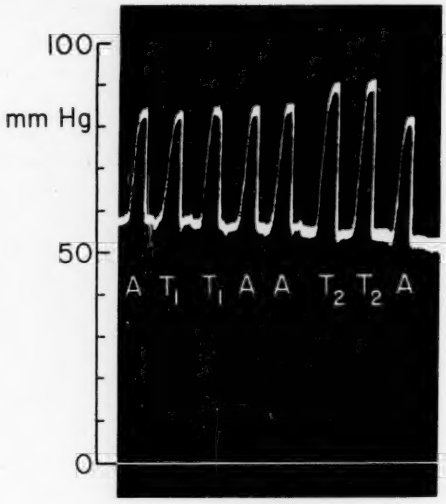


Fig. 30. Blood pressure of rat (305 g). Urethane. Dibenamine 2.44 mg. The pressor assay of the posterior pituitary gland of Rat HL 464, in which the lesions caused marked destruction of the supraoptic nuclei.

A = Hypadrin–standard (0.005 unit).
T_1 = 1/160 of the posterior pituitary gland.
T_2 = 1/100 of the posterior pituitary gland.

4. *Animals with lesions in the region of the supraoptic nuclei*

In the series with lesions in the region of the supraoptic nuclei, 3 animals with localized marked destruction of these nuclei had shown temporary polyuria (see Section A of this chapter). Two of the animals, HL 464 and 469, were killed about 3 months after the operation, when the daily urine output had returned to normal or almost normal levels.

The number of cells in the combined right and left supraoptic nuclei was 4 856 and 3 616, respectively. The corresponding figures for the paraventricular nuclei were 1 888 and 2 608 cells. The mean number of cells in the supraoptic nuclei of normal animals was 13 668 ± 483 (see chapter IV).

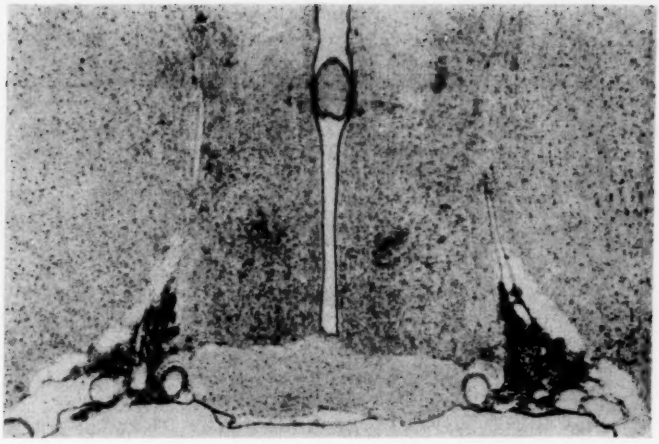
The amount of vasopressor activity in the posterior pituitary glands of the 2 rats HL 464 and 469 was found to be 0.8 and 0.6 unit, respectively, and that of oxytocic activity, determined as described in Section C of this chapter, 0.8 and 0.4 unit (Table 35). Part of the record of the vasopressor assay of HL 464 is given in Fig. 30, and Fig. 31 shows the location and extension of the hypothalamic lesions in this animal.

The amount of vasopressin in the posterior pituitary gland was, then, within normal limits, in spite of the great reduction of the supraopticoneurohypophysial system caused by the lesions.

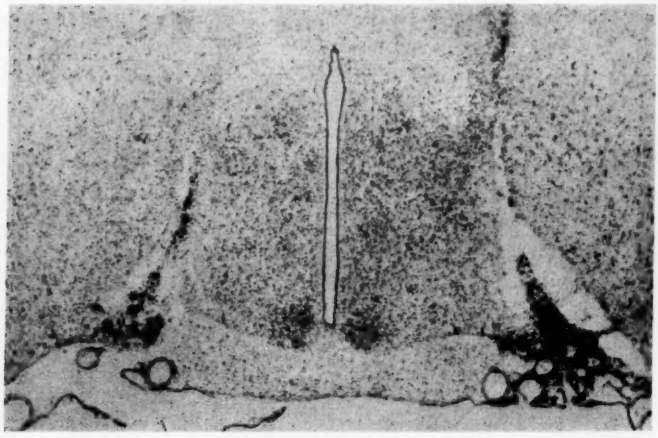
DISCUSSION

The posterior pituitary gland of the adult intact rat was found to contain 0.4 to 0.8 unit of antidiuretic-vasopressor hormone. This was in accordance with the finding of SIMON (1934), while DICKER & TYLER (1953 b) stated the amount of vasopressor activity to be about 0.35 unit.

Electrolytic lesions completely or partially destroying the paraventricular nuclei, like lesions located laterally to or behind these nuclei, caused little or no reduction in the amount of vasopressin in the posterior pituitary gland. Thus, the paraventriculoneurohypophysial system does not seem to be of importance for the formation of a normal amount of antidiuretic-vasopressor hormone. This result also fits in with the finding reported in Section A of this chapter, *viz.* that such lesions do not produce any disturbance in water metabolism referable to a deficient secretion of antidiuretic hormone. As shown by FISHER, INGRAM & RANSON (1938), the onset of the permanent phase of diabetes insipidus in the cat occurs about 10–13 days after the interruption of the nerve fibre connections between the supraoptic nuclei and the neurohypophysis. Posterior pituitary glands from such animals were found to be practically free from antidiuretic, vasopressor and oxytocic activity (FISHER & INGRAM, 1936, FISHER, INGRAM & RANSON, 1938). It may be assumed that the development of the permanent phase corresponds to the time at which the amount of antidiuretic hormone stored in the neurohypophysis has been consumed, which would thus be about a fortnight after

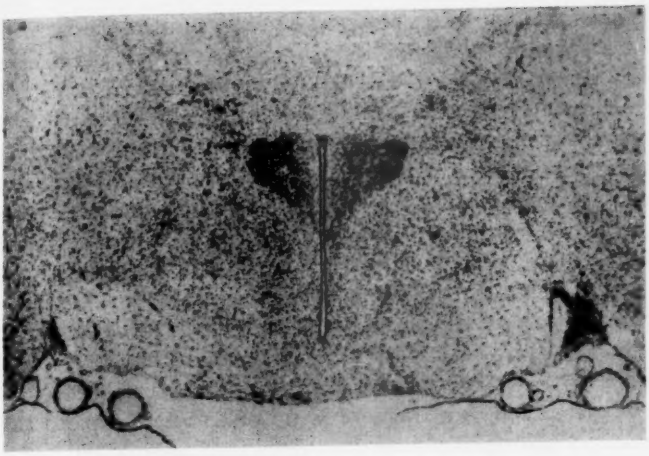


31 a. Section at a level through the anterior end of the optic chiasma.

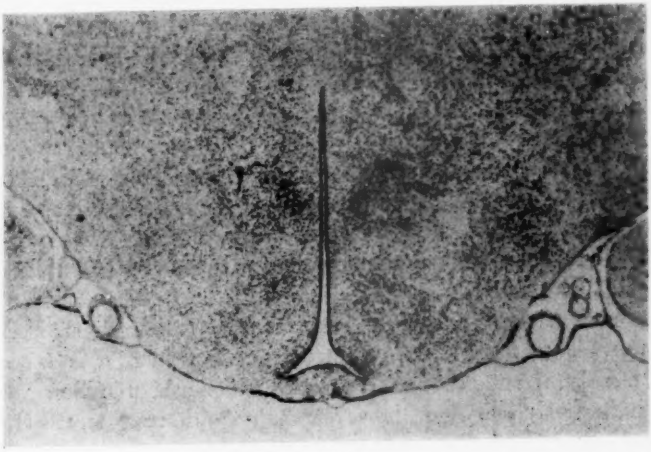


31 b. Section at the level of the suprachiasmatic nuclei.

Fig. 31 a–d. Transverse sections through the hypothalamus of Rat HL 464, which has a marked, direct destruction of the supraoptic nuclei. Gallocyanin stain. × 25.



31 c. Section at the level of the paraventricular nuclei.



31 d. Section at the level of the median eminence.

operation, leading to a subsequent increase in urine output. Most of the operated animals in the present investigation had received loads of hypertonic sodium chloride solution, and as increase in the osmotic pressure of the blood is known to increase neurohypophysial activity (VERNEY, 1946, 1947, 1948), it is possible that the posterior lobe was partly depleted of its content of antidiuretic-vasopressor hormone. If so, the approximately normal amount of vasopressin found on assay of the posterior pituitary glands would then be an expression of a new formation of the hormone even in the presence of the hypothalamic lesions.

The temporary disturbance in the fluid exchange in animals with marked destruction of the supraoptic nuclei was probably due to the cell loss in these nuclei. The polyuria should presumably not be regarded as a transient phase of diabetes insipidus since it was not followed by any permanent phase, and since the increase in water exchange started later than would have been expected had it been a real transient phase. The amount of vasopressor activity in the posterior pituitary gland was not determined during the polyuric period, but it seems probable that this was a manifestation of a decreased amount of antidiuretic-vasopressor hormone. By the time of the assay the polyuria had subsided, and the posterior lobe was found to contain an amount of vasopressin within normal limits.

A hypothetic explanation of this finding might be that, if the secretion of the antidiuretic-vasopressor hormone is linked only to the supraopticoneurohypophysial system in the strict sense of the term, a marked reduction of the size of the latter would accordingly lead to an acute decrease in the production of the hormone. If the release of the hormone during the first few weeks after operation exceeds the reduced new formation, a deficiency of circulating antidiuretic-vasopressor hormone should follow, with resulting polyuria. Since, however, the polyuric state ceased after some time, and the content of vasopressin in the posterior pituitary gland was at that time found to be within normal limits, this would mean that the remaining neurons in the reduced supraopticoneurohypophysial system were able to compensate the cell loss, and to produce both the amount of hormone necessary for the normal basal secretion and the surplus to be stored in

the posterior lobe, and demonstrable there on assay of extracts of the gland. The mechanism for the development of the polyuria in the present experiments might perhaps be still better explained on the assumption that the extensive destruction of the supraoptic nuclei produced by the lesions also caused a transient paralysis of the surviving cells, which then later recovered.

Inducing permanent diabetes insipidus after lesions in the region of the supraoptic nuclei therefore probably requires an almost complete destruction of these nuclei. Such an assumption would also be in agreement with the findings of FISHER, INGRAM & RANSON (1938), who showed that lesions in front of the median eminence, bilaterally interrupting the supraopticohypophysial tract, taken in the wider sense of the term, result in diabetes insipidus, while degeneration of only about half of the nerve fibres from the anterior hypothalamus does not cause any serious disturbance in fluid exchange.

SUMMARY

The determination of the amount of vasopressor activity in the posterior pituitary gland of rats with hypothalamic lesions in the region of the paraventricular nuclei showed that the paraventriculoneurohypophysial system is not necessary for normal formation of vasopressin.

The posterior pituitary glands of 2 rats with marked destruction of the supraoptic nuclei, causing a temporary disturbance in the fluid exchange, contained an approximately normal amount of vasopressin after this disturbance had subsided.

C. DETERMINATION OF THE AMOUNT OF OXYTOCIN IN THE POSTERIOR PITUITARY GLAND IN ANIMALS WITH TRANSECTION OF THE INFUNDIBULAR STEM OR WITH LESIONS IN THE REGION OF THE PARAVENTRICULAR NUCLEI

In the neurohypophysis of the adult rat the ratio of oxytocic activity to vasopressor activity has been found to be about 1 (DICKER & TYLER, 1953 b).

In Section B of this chapter it was shown that complete destruction of the paraventricular nuclei is compatible with a normal pressor content in the posterior pituitary gland.

This Section is concerned with the results of the determination of the oxytocic activity in the posterior lobes of rats with electrolytic lesions in the region of the paraventricular nuclei. Such determinations will provide some information on the importance of the paraventriculoneurohypophysial system for the normal secretion of oxytocin. Most of the glands assayed were also tested for their vasopressor activity. Further, determinations were made of the content of oxytocin in the posterior pituitary glands of animals with transection of the infundibular stem.

METHODS

PREPARATION OF EXTRACTS

In most of the cases the extraction was performed in 0.25 per cent acetic acid, as described in the foregoing Section, and the extract was assayed on the same day or the day after. These assays were thus performed without knowledge of the exact effect of the lesions on the various hypothalamic areas.

In the earlier cases, however, the acetone dried posterior lobes were extracted in 1/10 N hydrochloric acid, and the extract was

then stored in a deep-freezing box until the performance of the assay. Immediately before the test the extract was neutralized with 1/10 N sodium hydroxide solution.

As standard, use was made of extracts that had been prepared from pooled normal posterior lobes or from single, isolated normal glands, and secondly, of extracts prepared from the "Hypadrin" powder used as substandard.

Glands of operated animals extracted in 1/10 N hydrochloric acid and stored in frozen condition were tested against normal posterior lobes likewise extracted in 1/10 N hydrochloric acid. Also this standard extract was stored in frozen condition until used, and before the assay it was neutralized with 1/10 N sodium hydroxide solution.

DETERMINATION OF THE OXYTOCIC ACTIVITY

The isolated uterus of the virgin rat, usually in oestrus, was used for the assay of oxytocic activity (HOLTON, 1948).

Apparatus. – In the early assays the capacity of the organ bath used was 25 ml, while the major part of the tests were performed in a bath containing 10 ml. The surrounding water bath was kept at a constant temperature of about 32° C.

In order to minimize the tendency to spontaneous contractions, the uterus was suspended in the modified Locke's solution recommended for this purpose by DE JALON, BAYO & DE JALON (1945) and containing half the usual amount of glucose and a quarter of the usual amount of calcium. It has the following composition:

I.	NaCl	45 g
	KCl	2.1
	$CaCl_2(6H_2O)$	0.6
	Redistilled water	4.5 litres
II.	Glucose	2.5 g
	$NaHCO_3$	2.5
	Redistilled water	0.5 litre

Nine parts of solution I and one part of solution II were mixed. Solution II was freshly prepared.

One end of the uterus was fastened by a length of thread to a small hook at the lower end of a glass tube carrying air to the bath. The pressure of air was so adjusted that a stream of fine bubbles was produced. The other end of the uterus was fastened by a length of thread to a frontal writing lever arranged in such a way that the uterine contractions were magnified about 4 times.

Performance of the test. – The standard and test solutions were given with separate tuberculin syringes and at regular intervals of 5 minutes. The doses were given in series of 4, two of the standard and two of the test solution, injected into the bath in the order A B B A.

When the uterine contraction had subsided the bath was emptied, rinsed, and refilled with Locke's solution.

In the 10 ml bath doses corresponding to 1/80, 1/160 or less of a normal posterior pituitary gland gave adequate uterine responses.

In those cases where extracts of glands from operated animals did not give any response to the usual dose, the latter was successively increased so as to reach a maximum corresponding to 1/8 or 1/4 of the posterior lobe, implying the administration of 1 ml of the extract to the bath.

Unless otherwise stated the capacity of the isolated organ bath used was 10 ml.

RESULTS

NORMAL ANIMALS

In a series of 19 animals, 10 males and 9 females, with body weights ranging from 145 to 285 g (mean 215 ± 8.5 g), the amount of oxytocic activity in the posterior pituitary gland varied between 0.2 and 0.8 unit with a mean of 0.6 ± 0.05 unit (Table 36). No significant difference was found between male and female rats.

Assay of 43 normal posterior lobes showed that a dose corresponding to 1/80 or less of a gland gave rise to adequate uterine contractions, when use was made of a bath of 10 ml capacity.

Part of the record of the assay of a normal posterior pituitary gland against the "Hypadrin"-standard is given in Fig. 32.

Table 36. *The amount of oxytocin in the posterior pituitary glands of normal animals.*

Rat No.	Sex	Body wt g	Amount of oxytocin units
N 61	♂	220	0.4
63	♂	225	0.4
67	♂	225	0.8
69	♂	170	0.4
71	♂	225	0.2
73	♂	285	0.4
75	♂	220	0.6
151	♂	285	0.8
152	♂	250	0.8
153	♂	250	0.8
64	♀	145	0.8
68	♀	180	0.8
70	♀	165	0.5
72	♀	185	0.4
74	♀	200	0.8
147	♀	220	0.4
148	♀	220	0.8
149	♀	210	0.8
150	♀	210	0.8
Mean ± S.E.			0.6 ± 0.05

t-test					
Subject of the test	Males	Females	Difference	Degrees of freedom	P
Amount of oxytocin	0.56 ± 0.07	0.68 ± 0.06	−0.12 ± 0.10	17	> 0.1

ANIMALS WITH TRANSECTION OF THE INFUNDIBULAR STEM

This series consisted of 16 females with body weights varying between 125 and 230 g (mean 165 ± 6.5 g). The animals were killed 41–76 days after the operation.

Complete division of the hypophysial stalk with separation also of the pars tuberalis from its connection with the hypothalamus was not always achieved, as judged from the occurrence of normal ovary weights in some of the animals. This was, however, of less importance as the purpose of the operation was to produce a

complete interruption only of the nerve fibres from the hypothalamus to the infundibular process in their passage through the infundibular stem. Section of the stem results in a marked degeneration of the supraoptic and the paraventricular nuclei, and in 9 of the animals this was confirmed by determination of the number of magnocellular cells in these nuclei (see chapter IV).

All of the posterior lobes from the operated animals were extracted in 1/10 N hydrochloric acid. They were assayed against a standard extract (RS), prepared from the posterior pituitary glands of 74 normal animals. In these assays the capacity of the isolated organ bath was 25 ml.

A dose of the standard corresponding to 1/20 or 1/40 of a normal posterior pituitary gland gave satisfactory uterine responses. The posterior lobes from the animals in which the infundibular stem had been divided did not, however, show any oxytocic effect at all, even in doses as high as one fourth of a gland.

ANIMALS WITH LESIONS IN THE REGION OF THE PARAVENTRICULAR NUCLEI

EXTRACTION OF THE POSTERIOR PITUITARY GLAND IN 1/10 N HYDROCHLORIC ACID

Out of 12 animals, 11 males and 1 female, the paraventricular nuclei were completely destroyed in 7. In the other 5 animals the number of remaining magnocellular cells in these nuclei varied between 172 and 760. The animals were killed 128–241 days after the operation, and their body weights ranged from 155 to 315 g (mean 240 ± 15.8 g).

The posterior lobes of the operated animals were assayed against the RS-standard referred to above, or against a standard (St) prepared from the posterior lobes of 5 normal animals on the same day as the assay was performed. The RS-standard was used in those cases in which the posterior lobe extract had been stored in frozen condition, while the St-standard was used in those cases in which the test extracts were freshly assayed. Also in these assays the capacity of the bath used was 25 ml.

The posterior pituitary gland of each experimental animal was extracted individually. Just before the assay the extracts of glands

Fig. 32. Isolated rat uterus. Bath volume 10 ml. Assay of oxytocic activity of a normal posterior pituitary gland (N 69) against the Hypadrin-standard. Extraction performed in 0.25 per cent acetic acid.

from 6 animals were taken together in pairs (HL 270–272, 282–306, 289–293), while in the remaining cases each gland was tested separately.

The results of the assays are summarized in Table 37. The amount of oxytocin in the posterior lobes of operated animals is given as a percentage of that contained in the standard extracts used.

In the assays of HL 289–293 and HL 362, the dose of the standard corresponded to 1/10 and 1/80 of a normal posterior

Table 37. *The amount of oxytocin in the posterior pituitary glands of animals with electrolytic lesions completely or partially destroying the paraventricular nuclei. The glands were extracted in $^1/_{10}$ N hydrochloric acid.*

Rat No.	Sex	Days after op	Body wt g	Number of cells in the parav. nuclei	Oxytocic activity in per cent of standard
HL 270	♂	174	235	0	<20*
272	♂	174	275	0	
282	♂	174	300	0	<20*
306	♂	171	195	0	
277	♂	174	290	0	<20
299	♂	171	315	0	<40
289	♂	173	195	0	<40
293	♂	174	290	400	
362	♂	163	210	172	35
324	♂	241	170	296	10
325	♂	199	260	320	50
375	♀	128	155	760	100

* No uterine response to administration of one fourth of the posterior lobe.

pituitary gland, respectively, while in the remaining cases the standard dose was equivalent to 1/20 or 1/40 of a normal gland.

The addition of 1/4 of the posterior pituitary gland from the animals HL 270, 272, 282 and 306 to the uterine bath did not cause any contraction of the musculature. The posterior lobe of HL 277 did not show any oxytocic activity in a dose corresponding to 1/8 of the gland, while a small response was obtained after doubling this dose.

Fig. 33 shows the lack of any demonstrable amount of oxytocin in the combined posterior lobes from HL 282 and 306.

Four of the extracts assayed contained the posterior pituitary glands from 6 animals with complete destruction of the paraventricular nuclei, and in 5 of these glands there was a marked decrease in the amount of oxytocic activity.

In 2 animals, HL 324 and 325, both of which had about the same residue of magnocellular cells within the paraventricular nuclei, the posterior lobe of the former contained about 10 per cent of the amount of oxytocin found in the normal glands, while the corresponding figure for the latter was about 50 per cent.

The animal HL 375, which had the greatest number of surviving

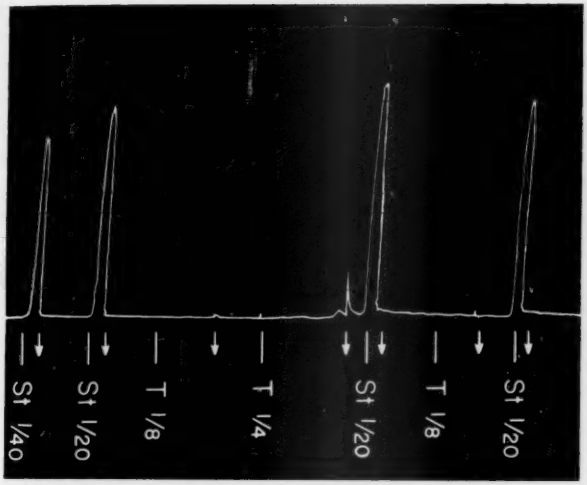


Fig. 33. Isolated rat uterus. Bath volume 25 ml. Assay of oxytocic activity of the combined posterior pituitary glands of Rats HL 282 and 306, in which the lesions caused complete destruction of the paraventricular nuclei. Extraction performed in 1/10 N hydrochloric acid.

St = Standard prepared from 5 normal posterior pituitary glands.
T = The combined posterior pituitary glands of HL 282 and 306.
The figures represent the dose expressed as part of a posterior pituitary gland. The arrows indicate emptying of the bath.

magnocellular cells, also showed the greatest amount of oxytocic activity in the posterior pituitary gland.

EXTRACTION OF THE POSTERIOR PITUITARY GLAND IN 0.25 PER CENT ACETIC ACID

1. *Animals with complete destruction of the paraventricular nuclei*

This series consisted of 11 animals, 8 males and 3 females. They were killed 129–573 days after the operation, and their body weights varied between 210 and 365 g (mean 270 ± 15.3 g).

The amount of vasopressor activity in the posterior pituitary gland had been determined in all of the animals except HL 341.

The posterior lobes of the operated animals were compared with St-standard, freshly prepared from 5 normal glands, with individual normal posterior lobes or with the "Hypadrin"-standard.

In 5 cases there was a marked decrease of the oxytocic activity in the posterior pituitary gland, while in the remaining 6 cases the amount of oxytocin was found to be within normal limits (Table 38). Part of the record of the assay of the posterior lobe from HL 366 showing a marked decrease in oxytocic activity is given in Fig. 34, and Fig. 35 shows part of the assay of the posterior lobe from HL 405 with an approximately normal amount of oxytocin.

In some of the animals with electrolytic lesions causing complete destruction of the paraventricular nuclei, there was thus simultaneously an approximately normal amount of vasopressin and a marked decrease in the amount of oxytocin in the posterior pituitary gland.

2. *Animals with partial destruction of the paraventricular nuclei*

In this series, which consisted of 23 animals, 13 males and 10 females, the amount of vasopressin in the posterior pituitary gland had been determined in 17 cases. The animals were killed 88–571 days after the operation, and their body weights ranged from 150 to 355 g (mean 255 ± 10.9 g). The number of remaining magnocellular cells in the combined right and left paraventricular nuclei varied between 112 and 1 884.

As standard the same types of extracts were used as for the animals in the series with complete destruction of the paraventricular nuclei.

In 2 animals, HL 326 and 431, with 620 and 864 magnocellular cells left in the paraventricular nuclei, respectively, there was a marked decrease in the amount of oxytocin in the posterior pituitary gland. In four animals, HL 338, 340, 376 and 404, in which only 112 to 268 magnocellular cells had escaped destruction by the lesions, the posterior lobes had an approximately normal content of oxytocin. In HL 356 the amount of oxytocin corresponded to

Table 38. *The amount of oxytocin in the posterior pituitary glands of animals with complete destruction of the paraventricular nuclei. The glands were extracted in 0.25 per cent acetic acid*

Rat No.	Sex	Days after op	Body wt g	Amount of vasopressin	Oxytocic activity in per cent of standard or in units
HL 366	♀	328	210	half the activity of a normal gland	< 5 %
313	♀	321	260	0.6 unit	<10 %*
332	♀	347	210	0.4 unit	<10 %*
311	♀	279	300	0.8 unit	<20 %**
337	♀	373	345	the same activity as in a normal gland	100 %
405	♀	129	225	the same activity as in a normal gland	200 %
415	♂	302	270	0.4 unit	0.05 unit
341	♂	472	365	—	0.4 unit
349	♂	573	270	0.8 unit	0.4 unit
407	♂	180	240	0.8 unit	0.5 unit
397	♂	339	270	0.8 unit	0.6 unit

* No uterine response to administration of one eighth of the posterior lobe.
** No uterine response to administration of one fourth of the posterior lobe.

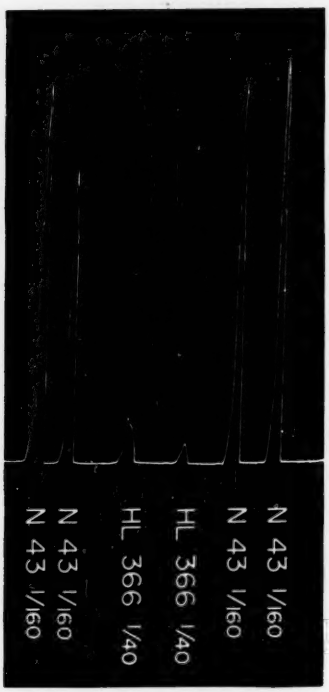


Fig. 34. Isolated rat uterus. Bath volume 10 ml. Assay of oxytocic activity of the posterior pituitary gland of Rat HL 366, in which the lesions caused complete destruction of the paraventricular nuclei. Extraction performed in 0.25 per cent acetic acid.

N 43 = Posterior pituitary gland of a normal animal.

The figures represent the dose expressed as part of a posterior pituitary gland.

0.2 unit, while in the remaining 16 animals the posterior pituitary gland contained 0.4–0.8 unit, or showed an activity equivalent to about the same or half that of normal glands (Table 39).

The posterior lobes from animals with partial destruction of the paraventricular nuclei had been found to contain about the normal amount of vasopressin, and in the present series the oxytocic

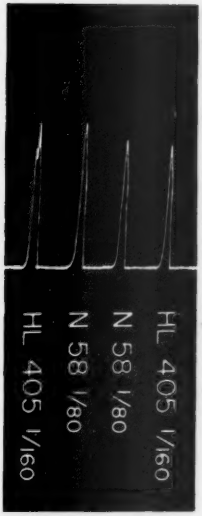


Fig. 35. Isolated rat uterus. Bath volume 10 ml. Assay of oxytocic activity of the posterior pituitary gland of Rat HL 405, in which the lesions caused complete destruction of the paraventricular nuclei. Extraction performed in 0.25 per cent acetic acid.

N 58 = Posterior pituitary gland of a normal animal.

The figures represent the dose expressed as part of a posterior pituitary gland.

assays of posterior pituitary glands from animals with similar lesions showed a marked decrease in the amount of oxytocin in 2 cases, while the content of this hormone in the remainder was within normal limits.

3. *Animals with lesions located laterally to or behind the paraventricular nuclei*

The results of the determination of the vasopressor activity in the posterior pituitary glands of the 7 animals belonging to this series were given in Section B of this chapter. The mean number of magnocellular cells in the combined right and left paraventricular nuclei was, as mentioned, $2\,265 \pm 196$.

Table 39. *The amount of oxytocin in the posterior pituitary glands of animals with partial destruction of the paraventricular nuclei. The glands were extracted in 0.25 per cent acetic acid.*

Rat No.	Sex	Days after op	Body wt g	Number of cells in the parav. nuclei	Amount of vasopressin	Oxytocic activity in per cent of standard or in units
HL. 326	♂♀	313	275	620	the same activity as in a normal gland	<20 %
431	♀	268	290	864	0.4 unit	<0.005 unit
315	♂♂	328	310	1024	0.6 unit	50 %
335	♂♂♂♂♀♀	366	255	812	half the activity of a normal gland	50 %
321	♂♂♂♀♀	366	290	564	the same activity as in a normal gland	65 %
373	♂♀♀	331	215	1024	half the activity of a normal gland	65 %
309	♂♂♂♂♀♀♀	277	340	1328	0.8 unit	100 %
384	♂♂♂♀♀♀♀	185	300	788	the same activity as in a normal gland	100 %
403	♀♀♀♀	114	215	488	the same activity as in a normal gland	100 %
404	♀♀♀♀	127	220	112	the same activity as in a normal gland	100 %
430	♀♀♀♀	88	150	1384	half the activity of a normal gland	100 %
377	♀♀♀♀	334	215	528	the same activity as in a normal gland	125 %
376	♀♀♀♀	345	240	268	0.5 unit	200 %
356	♀♀♀	571	190	616	0.4 unit	0.2 unit
338	♂♂♂	472	305	176	—	0.4 unit
347	♂♂♂♀♀	569	265	344	0.8 unit	0.4 unit
411	♀♀	176	245	612	—	0.4 unit
392	♂♂♂♀♀	358	270	1332	0.8 unit	0.4 unit
351	♂♀♀	510	160	1884	—	0.4 unit
340	♂♂♂	472	260	200	—	0.8 unit
339	♂♂♂	472	245	704	—	0.8 unit
396	♂♂♂♂♂	366	300	1032	0.8 unit	0.8 unit
342	♂	472	355	1184	—	0.8 unit

Table 40. *The amount of oxytocin in the posterior pituitary glands of animals with lesions located laterally to or behind the paraventricular nuclei. The glands were extracted in 0.25 per cent acetic acid.*

Rat No.	Sex	Days after op	Body wt g	Location of the lesions	Number of cells in the parav. nuclei	Amount of vasopressin units	Amount of oxytocin units
HL 353	♀	561	130	Lateral to the parav. nuclei	1600	0.4	0.4
357	♀	573	230	»	1712	0.8	0.3
441	♀	284	215	»	2052	0.8	0.8
440	♀	274	215	Behind the parav. nuclei	2180	0.8	0.8
439	♀	267	225	»	2600	0.8	0.8
446	♂♀	288	275	»	2796	0.4	0.4
437	♀	249	250	»	2912	0.8	0.8
Mean ± S.E.					2265 ± 196	0.7 ± 0.07	0.6 ± 0.09

In all the cases the posterior lobes were assayed against the "Hypadrin"-standard, and the amount of oxytocic activity in the glands was found to vary between 0.3 and 0.8 unit, with a mean of 0.6 ± 0.09 unit (Table 40).

In animals with such lesions, then, the amount of vasopressin as well as the amount of oxytocin was within normal limits.

ANIMALS WITH LESIONS IN THE REGION OF THE SUPRAOPTIC NUCLEI

The posterior pituitary glands of 2 animals, HL 464 and 469, in which the lesions had caused marked destruction of the supraoptic nuclei, were assayed for their amount of vasopressin as well as of oxytocin.

An account of the data of the animals, and the results of the assays of oxytocic activity are given in Table 35 in Section B of this chapter. It is apparent from this table that the posterior lobes from these 2 animals contained an approximately normal amount of oxytocic activity.

DISCUSSION

The results of the assays of the atrophied posterior pituitary glands from animals with transection of the infundibular stem confirmed the finding of Fisher & Ingram (1936) that interruption of the nerve fibres running to the posterior lobe is followed by loss of oxytocin in this part of the neurohypophysis.

After hypothalamic lesions a reduction in the amount of oxytocin in the posterior lobe was seen only after lesions in the region of the paraventricular nuclei, while in animals with lesions located laterally to or behind these nuclei, or causing a direct marked destruction of the supraoptic nuclei, the oxytocic activity of extracts of the glands was found to be within normal limits. This might suggest the paraventriculoneurohypophysial system to be of importance for the normal elaboration of oxytocin.

In the present investigation the extent of the paraventricular nucleus was always confined, both qualitatively and quantita-

tively, to its compact part only, while the scattered portions of magnocellular cells forming an irregular chain linking the lateral part of the paraventricular nucleus with the supraoptic nucleus, were not taken into account. Any attempt to destroy also these cells, which have been taken together under the common name of the pars lateralis of the paraventricular nucleus (BODIAN & MAREN, 1951), would have required such large lesions in the anterior hypothalamus that it would no longer have been possible to analyze the effect of a loss of the paraventriculoneurohypophysial system alone on the hormones of the pituitary gland. However, the possibility cannot be excluded that also nerve fibres from the magnocellular cells in the pars lateralis run to the neurohypophysis, and that further these neurons may have the same function as those extending from the principal part of the nucleus. If, therefore, under such circumstances, the pars lateralis of the paraventricular nucleus contains a sufficiently large number of magnocellular cells, it might be assumed that these would, even in the absence of the cells in the compact part of the nucleus, be able to maintain the function normally performed by the entire paraventriculoneurohypophysial system. That the various fibre systems in the entire hypothalamoneurohypophysial system probably possess a pronounced functional reserve capacity is apparent from the statement of FISHER, INGRAM & RANSON (1938) that only lesions causing bilateral interruption of the supraopticohypophysial tract with an almost total disappearance of cells in the supraoptic nuclei are capable of producing diabetes insipidus in the cat. Degeneration of half of the fibres of the tract, on the other hand, does not cause any disturbance in fluid exchange. Further evidence pointing in the same direction is the result of the determination of the amount of vasopressin in the posterior lobes of animals with marked, direct destruction of the supraoptic nuclei, and presented in the foregoing Section of this chapter. Despite the great loss of neurons within the supraopticoneurohypophysial system, taken in the strict sense of the term, the amount of vasopressin in the posterior lobe was within normal limits.

The existence of such a reserve of magnocellular neurons might explain the varying results obtained from the assays of the amount of oxytocin in the posterior pituitary glands from animals with

lesions located in the region of the paraventricular nucleus with complete destruction of its compact part.

In the present experiments the animals were allowed to live for rather a long time after the operation before they were killed. Hypothetically, it therefore seems possible that in analogy with the assumption as regards the elaboration of the antidiuretic hormone by a greatly reduced supraopticoneurohypophysial system, the spared magnocellular cells belonging to the pars lateralis of the paraventricular nucleus might, during this period, have been able to produce not only the amount of oxytocin necessary for the normal basal secretion, but also that excess stored in the posterior pituitary gland and there determinable on oxytocic assay.

In the earlier series in which the posterior lobes from animals with complete destruction of the paraventricular nuclei were extracted in hydrochloric acid, the lesions involved a greater part of the surrounding structures than in the later series, and this might be the cause of the greater number of animals with marked decrease in the amount of oxytocin found in the former as compared with the latter.

The observation that, in posterior pituitary glands from animals with lesions in the region of the paraventricular nuclei, there could be a marked decrease in the amount of oxytocin in the presence of an approximately normal amount of vasopressin, seems to suggest that the antidiuretic-vasopressor and oxytocic hormones may exist as separate entities physiologically.

It therefore appears possible that the paraventriculoneurohypophysial system – and it might be that this should be taken in the wider sense of the term including also the scattered neurons belonging to the pars lateralis of the paraventricular nucleus – participates in the regulation of the secretion of oxytocin.

It is generally believed that the supraoptic and paraventricular nuclei, which in lower vertebrates are represented by one common nucleus, the nucleus preopticus (Meyer, 1935), also have a common function, both being concerned with the elaboration of the two posterior pituitary principles (see Introduction to this chapter). If this be the case, it cannot be expected that destruction solely of the paraventricular nuclei, which contain only about 16 per

cent of the total number of cells belonging to the hypothalamic magnocellular nuclei, should be followed by any signs of deficient secretion of these hormones from the neurohypophysis.

In Section A of this chapter it was shown that complete, localized destruction of the paraventricular nuclei did not cause any disturbance in water metabolism attributable to a diminished amount of circulating antidiuretic hormone, while marked, direct destruction of the supraoptic nuclei without any involvement of the paraventricular nuclei resulted in temporary polyuria.

This finding taken together with the results of the oxytocic assays, reported in this Section, provide some reason for suspecting the two main fibre systems within the total hypothalamoneurohypophysial system to be not only anatomically separated, but also functionally different, the paraventriculoneurohypophysial system thus being concerned with the control of oxytocic secretion and the supraopticoneurohypophysial system with that of the secretion of antidiuretic-vasopressor hormone.

SUMMARY

The results of assays of posterior pituitary glands from animals with hypothalamic lesions located in the region of the paraventricular nuclei suggested the paraventriculoneurohypophysial system to be of importance for the normal elaboration of oxytocin. The possibility that this fibre system should be taken in the wider sense of the term including also the scattered magnocellular neurons belonging to the pars lateralis of the paraventricular nucleus is discussed.

The decrease in the amount of oxytocin occurred independently of the vasopressor content of the posterior lobe, suggesting the two hormones to exist as separate entities physiologically.

GENERAL DISCUSSION

The question whether the supraoptic and the paraventricular nuclei have one and the same or separate functions has not hitherto received close attention. The similarity in cytologic respect as well as the conformity in the mode of reaction on the part of the nerve cells to conditions placing a load on neurohypophysial function (cf. HILLARP, 1949 b), has been regarded as evidence in support of the belief that the two nuclei have a common function. Further, experiments aiming at separate electric stimulation of these nuclei – although giving fairly discordant results (see Introduction to chapter VI) – seem also to some extent to support such an opinion. Very strong evidence, speaking in favour of such a view, must, however, be brought forward before we accept a common function in two anatomically distinct neuron groups. As it may be difficult to interpret the results obtained with stimulation technique (see below), it was considered more convenient to make an analysis of the effect of localized destruction of only one of these two nuclei at a time in order further to be able to elucidate this problem.

In any attempt to refer specific functions to certain distinct cell groups in the hypothalamus with the use of experimental electrolytic lesions it seems absolutely necessary to take into consideration two essential demands: the lesions should be circumscribed, *i. e.* involving as little as possible of the surrounding nuclear areas, and secondly, the effects produced by the lesions should be compared with those of other lesions located in the immediate vicinity of the cell group to be examined. As regards the supraoptic nuclei it seems difficult to satisfy these demands, which, however, as far as the paraventricular nuclei are concerned, could be met in the present investigation, at least in part. A third important requirement to be fulfilled in experimental work on the hypothalamoneurohypophysial system is the necessity of

avoiding involvement in the lesions of nerve fibres in front of the median eminence, located mediobasally under the floor of the third ventricle. This latter question has been discussed in Section A of chapter V and in the Introduction to chapter VI.

It should be borne in mind that the paraventricular nucleus contains two different cell types, the magnocellular and the parvocellular nerve cells, and that a destruction of the nucleus will involve both groups. Since, however, there is no evidence that the parvocellular cells send their axons to the neurohypophysis, and since these cells – in contrast to the magnocellular cells, the axons of which are in connection with the neurohypophysis – lack neurosecretory material, it does not seem probable that they are directly concerned with the formation and secretion of posterior pituitary hormones. In the following discussion of the significance of the paraventricular nuclei for posterior pituitary function it will therefore be regarded as most likely that this can be confined to comprise only the magnocellular portion of the nucleus.

The evidence for and against the existence of two separate posterior pituitary hormones has been discussed in the Introduction to chapter VI. Several experimental findings appear to speak in favour of the view that the oxytocic and the antidiuretic-vasopressor principles represent two separate hormones. Such an assumption is strongly supported by the observation in the present investigation that complete destruction of the paraventricular nuclei may result in a pronounced decrease of the oxytocic activity of the posterior pituitary gland, without affecting its amount of vasopressor activity. It therefore seems most reasonable to suppose the elaboration of at least two separate hormones by the hypothalamoneurohypophysial system. On account of this finding, and since the neurohypophysis is in connection with two anatomically distinct magnocellular nuclei, it seems an attractive hypothesis to assume each nucleus to be concerned with the formation and secretion of only one of the two posterior pituitary hormones.

The fact that destruction of the paraventricular nuclei, which contain only about 16 per cent of the total number of the hypothalamic magnocellular cells, may result in a pronounced diminishing of the amount of oxytocic activity in the posterior

pituitary gland, strongly suggests that the oxytocic hormone is elaborated by the paraventriculoneurohypophysial system. Unfortunately, it was not possible to produce a complete, localized destruction of the supraoptic nuclei. The present experiments did, however, lend some support to the assumption that such lesions will be followed by a state of diabetes insipidus, indicating loss of circulating antidiuretic hormone. If so, the elaboration of this hormone would thus be confined to the supraopticoneurohypophysial system.

The possibility of eliciting a release of both antidiuretic and oxytocic hormones by electric stimulation of either the supraoptic or the paraventricular region (ANDERSSON, 1951 b, ANDERSSON & MCCANN, 1955 a and c), however, militates against the above-mentioned hypothesis. The results of such experiments are, however, – as also pointed out by ANDERSSON – difficult to interpret. Firstly, no quantitative data concerning the amount of hormones secreted are available. Secondly, – and what is most important – it is not possible to exclude with any degree of certainty that stimulation of the paraventricular nuclei does not cause a simultaneous stimulation of nerve cells or nerve fibres being in connection with the supraoptic nuclei (the parvocellular cells of the paraventricular nucleus may, for instance, represent such a connection), or that stimulation of the supraoptic nuclei does not cause a simultaneous stimulation of nerve fibres running to the paraventricular nuclei, or of axons extending from these latter nuclei to the neurohypophysis.

The question may be raised why complete destruction of the paraventricular nuclei do not regularly result in a disappearance of oxytocin in the posterior pituitary gland. As discussed in Section C of chapter VI this may be due to the existence of uninjured magnocellular cells located in the region between the paraventricular and the supraoptic nuclei, having the same function as those forming the former cell group. It is, however, possible to propose another, and presumably more reasonable explanation of the divergency of the results obtained.

As pointed out above it seems most plausible to assume the hypothalamoneurohypophysial system to constitute the site of formation of at least two different posterior pituitary hormones.

Several experiments have furthermore produced evidence suggesting the oxytocic and antidiuretic hormones to be secreted more or less independently of each other (see Introduction to chapter VI). However, it appears to be difficult to understand how such a differentiation in function would be effected on the assumption of only one type of nerve cells in the hypothalamic magnocellular systems. It therefore seems more likely to suppose the occurrence in these two fibre systems of two different cell types. The neurons concerned with the elaboration of the oxytocic hormone would then mainly belong to the paraventricular nuclei, while those engaged in the formation of the antidiuretic-vasopressor hormone would be located in the supraoptic nuclei. The complete separation of the postulated two cell types into two anatomically different structures may, however, be more or less imperfect in the individual case. Such a hypothesis would provide an explanation not only of the results obtained in the present work, but also of the results of those experiments in which electric stimulation of the magnocellular hypothalamic nuclei was used.

More direct evidence of the correctness of the advanced hypothesis cannot be provided, however, by stimulation or lesion techniques. The demonstration of any possible functional differentiation of the morphologically uniform-looking magnocellular cells within the paraventricular and supraoptic nuclei will require the working out of cytochemical methods for direct demonstration of the hormone peptides in the nerve cells in analogy with the histochemical demonstration of adrenaline and noradrenaline secreting cells in the adrenal medulla.

ACKNOWLEDGEMENT

The investigation was supported by grants from the Faculty of Medicine, University of Lund.

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